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QUARTERLY JOURNAL
OF
MICROSCOPICAL SCIENCE.

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By

Gilbert C. Bourne, M.A., D.Sc., F.R.S.,
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All students of microscopical science and his numerous friends, colleagues and pupils will unite in offering hearty congratulations and cordial expressions of esteem and goodwill to Sir Edwin Ray Lankester, who for fifty years has been the editor of the 'Quarterly Journal of Microscopical Science.' Equally all will agree in wishing him many more years of health and energy in which he may continue his invaluable labours in the cause of zoological science.

The 'Quarterly Journal of Microscopical Science' was started in 1853 with Mr. Samuel Highley as publisher. The publisher's business was transferred in 1856 to Mr. John Churchill, and the two original editors, Dr. Edwin Lankester and Mr. George Busk, continued to carry on the Journal up to the end of 1868.

The introduction to the first volume of the old series tells us that the object of the Journal was "the diffusion of information relating to all improvements in the construction of the microscope, and to record the most recent researches made by its aid in different departments of science, whether in this country or on the Continent. A department of the Journal will be given to reviews of works . . . In order to gather up fragments of information which singly might appear to be useless but together are of great importance to science, the editors have opened a department for short notes, memoranda and correspondence."

The original scope of the Journal, therefore, was wider than it is now, and the earlier volumes, in addition to memoirs on zoological and botanical subjects, contain many papers on the construction and theory of the microscope and on improvements in microscopical technique. By agreement with the Microscopical Society of London the Transactions of that Society were included and published in the same volumes as the 'Quarterly Journal of Microscopical Science,' and this arrangement continued up to the end of 1868, when the Royal Microscopical Society, having recently (1866) been incorporated by Royal Charter, decided to sever its connection and to publish its transactions independently in its own periodical, the 'Monthly Microscopical Journal.' Mr. George Busk retired in 1868 from the co-editorship of the 'Quarterly Journal,' and in 1869 E. Ray Lankester, then a newly graduated B.A. of Oxford, joined his father in the editorship.

The old series of the 'Quarterly Journal of Microscopical Science' comprised eight volumes, from 1853 to 1860, both years inclusive. In 1861 a "new series" was instituted, and the volumes bear consecutive numbers from that date to the present day. It was the ninth volume of the new series which started on an independent career under the auspices of the two Lankesters, father and son. Dr. Edwin Lankester retired in 1872 and, at Ray Lankester's request, Dr. J. F. Payne, a medical graduate of Oxford and Fellow of Magdalen College, came to his aid. In 1873 Lankester's fellow-student and life-long friend joined him as editor—namely W. T. Thiselton Dyer, who later became Director of the Royal Gardens at Kew. He retired in 1876 and Mr. William Archer, of Dublin, was invited by Lankester to be one of the editors. In 1877 Dr. Payne retired, and Ray Lankester sought and obtained the co-operation of an old friend, Dr. Klein, with whom he had been associated in Stricker's laboratory at Vienna. In 1878 the editorial arrangements were modified. Ray Lankester became the sole editor, and the names of W. Archer, Francis Maitland Balfour and E. Klein appear on the title-page as acting in co-operation with him. The policy which has contributed so much to the influence and success of the Journal was then determined and has been steadily adhered to. Sir Ray's object was to attract the best work of rising men from Oxford, Cambridge, London, Edinburgh and other centres, and for this reason he invited the co-operation of the leading teachers in the several Universities. There have been many changes in the list of co-operators. The hand of death has fallen heavily on British zoologists, and the names of several who, like F. M. Balfour, had established great reputations at an early age, disappear all too soon from the title-pages, their places being taken by others. In addition to those mentioned above Adam Sedgwick, H. N. Moseley, A. Milnes Marshall, W. F. R. Weldon, S. J. Hickson, E. A. Minchin, G. C. Bourne, J. Graham Kerr and E. W. MacBride have at various times co-operated with Sir Ray Lankester in the editing of the Journal. Their share has been to supply fresh contributors from among their best pupils; the whole responsibility and work of editorship has been undertaken by the chief editor. The hospitality of the Journal has always been open to naturalists from other countries. The late Prof. E. van Beneden contributed some of his earliest memoirs on Gregarina, Dicyemidæ and embryology to the Journal between 1870 and 1878. Among the American zoologists we notice contributions from W. B. Scott and H. F. Osborn in 1879 and 1881, A. S. Packard (1871 and 1872), C. O. Whitman (1878–1886), E. B. Wilson (1881), and E. Phelps Allis (1905 and 1917). The rising school of zoologists in Japan are represented by memoirs written by T. Ijima (1882), C. Ischikawa (1885), T. Iwakawa (1882), and K. Mitsukuri (1881). Of Belgian naturalists, in addition to E. van Beneden, we have contributions from M. L. Dollo (1883) and P. Pelseneer (1885 and

1894). France is represented by memoirs from A. Giard (1880), L. Ranvier (1880), and E. L. Bouvier (1900). From Holland we have papers by R. Horst (1882) and J. C. Oudemans (1885), whilst Prof. A. A. W. Hubrecht was a regular contributor, beginning in 1875 with the first of a valuable series of papers on Nemertea, and following up with another and even more important series on mammalian embryology extending from 1889 to 1910. From Italy we have the extremely interesting memoirs on the natural history of Termites by Grassi and Sandias in 1896 and 1897. The German naturalists are largely represented in translations and reviews, but the only original contributions of importance seem to be those of O. Bütschli in 1879, and R. von Erlanger, on the paired renal sacs of Prosobranchs, in 1891.

As was inevitable the scope of the Journal has changed considerably in the last fifty years. In 1869 it retained its original features, save for the disappearance of the transactions of the Royal Microscopical Society. Among the original memoirs botany was almost as largely represented as zoology, students of Diatomaceæ, among whom Henfrey, Gregory, Hendry, O'Meara, Donkin and Wallich may be mentioned, making special use of its pages. In other departments of botany S. H. Vines, F. Darwin, F. O. Bower and Walter Gardiner were for some years steady contributors, but the growth of specialisation in science led to the publication of the 'Journal of Botany,' and after the early eighties of last century but few botanical memoirs have appeared in the Journal, save for the contributions of bacteriologists and the important cytological memoirs of J. B. Farmer (vol. 48, 1905). The notes, memoranda and reviews which occupied so considerable a space in the decade following 1869 began to become scantier towards its close, and finally disappeared in 1883, at the same time that the size of the Journal was increased to medium octavo.

A Quarterly Chronicle of Microscopical Science containing much useful miscellaneous information was introduced in 1871 and formed a special department of the Journal for the next four years, but disappeared in 1875. For many years short notices of the transactions of various microscopical societies, particularly those of the Dublin Microscopical Club, were regularly recorded, but appear for the last time in vol. 20 (1881.)

It may be said that in 1883 the Journal had finally reached its present form and very nearly its present size. The contents, with the exception of occasional reviews or translations of foreign memoirs of special importance, are original memoirs on bacteriology, and the embryology, histology, bionomics and comparative anatomy of animals. A large and increasing space has been devoted to the progressive department of Protozoology.

As regards size, it may be recorded that vol. 23, the first to appear in medium octavo, contained 653 pages of letterpress and forty-three lithographic plates. By 1885 the number of contributors had so far increased as to require a supplement to the four quarterly numbers, and the volume is enlarged to 752 pages of letterpress and fifty-two lithographic plates. In 1890, owing to the increasing number of memoirs contributed, the strict routine of quarterly publication was abandoned and the numbers were published at shorter and less regular intervals, though, as before, four numbers went to make up a volume. Thus in twenty-eight years thirty-three volumes have been published.

If now we turn to the character of the memoirs that have appeared in the Journal in the course of fifty years we find that the later ones are generally longer and more abundant in detail than those in the earlier volumes. It can hardly be maintained that they are of greater intrinsic value. Many of the old memoirs contained pioneer work, and opened the way for the more copious treatment given to the same subjects in later years. But if the opportunities for pioneer work are less numerous nowadays—so much ground has been explored since 1869—the recent volumes of the Journal contain not a few memoirs which herald new departures in biological research, and on the other hand there are memoirs of forty years ago that leave little to be desired in the point of elaboration of detail.

At the head of the list of contributors stands Sir Ray Lankester himself. He is responsible for seventy-eight memoirs, including one published in the present year in the last volume, and all but three of these stand in his sole name. Their titles bear witness to the catholicity of interest so faithfully reflected in the management and character of the Journal. His earliest memoir, in new series, vol. 3 (1863), is "On our Present Knowledge of the Gregarinidæ, with Descriptions of Three New Species belonging to that Class." In succeeding volumes there are numerous descriptions of Protozoa from his pen, among which special mention may be made of the papers on Undulina (vol. 11, 1871) and Drepanidium ranarum (vol. 22, 1882). As will appear later on, this early interest in Protozoology and the lead given by the editor culminates in a series of very important memoirs by various authors. In 1864 and 1865 (vols. 4 and 5) Sir Ray made his second venture in zoological authorship by writing on the anatomy of the earthworm. These two memoirs were followed up in later years by his colleagues and pupils, F. E. Beddard, A. Gibbs Bourne and W. B. Benham, in numerous memoirs of monographic character, and their work is expanded in exquisite detail by the series of researches by E. S. Goodrich on the nephridia of the Polychæta.

In 1874 Sir Ray Lankester published his observations on the development of the pond-snail and on the early stages of other Mollusca—the first of a series from his pen which laid the foundations of the study of Molluscan embryology. His further researches on Mollusca are published elsewhere. A notable contribution to Molluscan anatomy is that of his friend and pupil, R. Holman Peck, whose memoir on the minute structure of the gills of Lamellibranch Mollusca (vol. 17, 1877) was written under the personal superintendence of the Editor. The memoir on the olfactory organ and paired genital ducts of the Pearly Nautilus, by Lankester in conjunction with A. G. Bourne (vol. 23, 1883), is another valuable contribution to the same subject.

In 1869, the year in which Sir Ray Lankester first took up editorial duties, Dr. G. J. Allman described Rhabdopleura, a new form of Polyzoa from deep-sea dredging in Shetland (vol. 9). With unerring instinct for what is not merely rare and curious, but also of fundamental morphological importance, Sir Ray Lankester kept this animal in mind, and we find a memoir on its affinities in vol. 14 (1874) and ten years later, in vol. 24, as the result of a visit to the Norwegian fiords, a remarkable and beautifully illustrated memoir describing the structure and habit of Rhabdopleura in great detail. Nine years later this interest in a curious and seemingly aberrant form was completely justified by G. H. Fowler's discovery that it is allied to the Hemichordata and so stands at the base of the vertebrate phylum (see vol. 48, 1904).

The series of articles by Sir Ray Lankester, beginning in vol. 21 (1881) with the memoir, "Limulus an Arachnid," are now among the classics of zoological literature, and to this day stand as an example of the way in which a morphological problem may be followed up in detail by critical analysis of every organ in the bodies of the animals brought into comparison. The paper on the minute structure of the lateral and central eyes of Scorpio and Limulus was written in conjunction with A. G. Bourne, and marks a great advance in our knowledge of the structure and genesis of the Arthropod eye. In later years (vol. 48, 1904) these memoirs were summed up and extended in a masterly review of the structure and classification of the Arachnida. As a parallel piece of research we may notice Lankester's illuminating memoir, "Observations and Reflections on the Appendages and on the Nervous System of Apus cancriformis," vol. 21 (1881), followed by P. Pelseneer's more detailed study of the nervous system of the same species (vol. 25, 1885), and the whole subject of Arthropod structure and classification is summed up in Sir Ray's essay in vol. 47 (1904), to which G. H. Carpenter's notes on the segmentation and phylogeny of the Arthropoda in vol. 49 (1905) is a fitting pendant.

To go back again to earlier days. Sir Ray Lankester from the first was deeply interested in the blood and the colouring matters of animals. We may note his early essay on the action of Monads in producing colouring matters (vol. 7, 1867), and another on blue stentorin, the colouring matter of Stentor cœruleus (vol. 13, 1873). His interest extended itself to his fellow-student, H. N. Moseley (vol. 17, 1877), whose paper on actiniochrome appeared in the volume last cited. The subject of animal chromatology received extended treatment at the hands of C. A. MacMunn in vols. 25, 27, 30 and 43. Nor should W. B. Benham's interesting observations on the blood of Magelona (vol. 39, 1896) be forgotten in this connection. The interest in colouring matters naturally extends to the occurrence of chlorophyll in the animal kingdom, and there is an early paper by Sir Ray Lankester in vol. 14 (1874) on the mode of occurrence of chlorophyll in Spongilla, followed eight years later by an illuminating memoir on the chlorophyll corpuscles and amyloid deposits of Spongilla and Hydra in vol. 22.

Space forbids a full appreciation of the numerous contributions to zoological science published by Sir Ray Lankester in the 'Quarterly Journal of Microscopical Science,' but no enumeration would be complete without special mention of his work on Amphioxus lanceolatus. As early as 1875 (vol. 15) he described some new points in the structure of this species and in particular his discovery of the brown atrio-cœlomic funnels. Fourteen years later, in vol. 29, appeared his "Contributions to the Knowledge of Amphioxus lanceolatus," a monographic description of the anatomy of this species which greatly extended our knowledge and enlarged our interest. In vol. 31 there followed an account of the development of the atrial chamber of Amphioxus, written in conjunction with A. Willey, and this led directly to Willey's studies on the later larval development of Amphioxus, vol. 32, and to his further studies on the Protochordata, vols. 34, 35 and 42. The running has been taken up by E. W. MacBride in his papers on the development of Amphioxus, vols. 40, 43 and 54, and by E. S. Goodrich by his demonstration that the supposed nephridial funnels are in fact solenocytes (vol. 45). Goodrich's further observations on Hatschek's nephridium and the nephridium in the larva, vol. 54, made a further and very notable contribution to our knowledge of this interesting species. In fact, Amphioxus has been quite a speciality of the Journal.

But, as anyone who searches through the volumes of the last fifty years will realise, though the 'Quarterly Journal of Microscopical Science' has had its specialities it has never been specialised. There have been not one speciality but many specialities. It is not possible to do justice to all of them, but attention may be called to notable advances made by notable men in various departments of zoological work.

One of the most important papers published in the earlier years of the fifty under review is the classical account by Mr. A. P. Thomas of the life history of the Liver Fluke and his discovery of Lymnæa truncatula as its intermediate host (vol. 23, 1883). Mr. Thomas carried out his investigations at Oxford under the direction of Professor Rolleston and succeeded in a quest in which Rudolph Leuckart had failed.

In the early seventies of the nineteenth century the remarkable researches of the Russian naturalist Kowalewsky, to which the attention of Englishmen was called by a notice in vol. 10 written, at Ray Lankester's request, by the late Sir Michael Foster, gave a marked impetus to embryological studies in this country. The beginning of a definite epoch is marked by the appearance of Francis Maitland Balfour's memoir on the development and growth of the germ-layers of the blastoderm in vol. 13 in 1873. This volume contains two other papers on the development of the chick written by the same hand, and in the following year, in vol. 14, Balfour's masterly memoir entitled "A Preliminary Account of the Development of the Elasmobranch Fishes" makes its appearance. Other memoirs followed in rapid succession, and vols. 19 and 20 contain no less than seven memoirs by Balfour, and an eighth on the existence of a head kidney in the embryo chick which was the joint production of himself and his pupil Adam Sedgwick. Two of these memoirs deserve special notice—the one on the spinal nerves of Amphioxus, because it was a contribution to a subject that has figured so largely in the pages of the Journal, the other on certain points in the anatomy of Peripatus capensis, because it was the first of a series no less famous in the annals of the Journal than that relating to Amphioxus. Another remarkable memoir by Balfour in vol. 20 is that on larval forms, their nature, origin and affinities. This same vol. 20 contains two papers on the development of the kidney in the chick by Adam Sedgwick, Balfour's pupil, successor and scientific executor. In 1882 Balfour lost his life by an accident in the Alps, and the memoir on which he was engaged on the anatomy and development of Peripatus capensis was published posthumously in vol. 23 (1883). At this time A. Sedgwick, partly as a tribute to the memory of his friend and teacher, made an expedition to South Africa to collect the embryos of Peripatus, and his results are set forth in four successive memoirs on the development of Peripatus capensis in vols. 25, 26, 27 and 28. In the last-named volume appears Sedgwick's monograph on the species and distribution of the genus Peripatus—a work originally undertaken as a tribute to Balfour's memory in conjunction with H. N. Moseley, but owing to the latter's illness it was completed by Sedgwick alone. Since that time important contributions to our knowledge of the genus have been published in the Journal by Lilian Sheldon, W. L. Sclater, E. L. Bouvier, Muir and Kershaw, E. C. Pollard and R. Evans.

Another famous addition to embryological science was made by another of Balfour's pupils, W. Bateson, whose three memoirs on the development of Balanoglossus kowalevskii, followed by an essay on the ancestry of the Chordata, are to be found in vols. 24, 25 and 26 of the

Journal. A third pupil of Balfour's, W. F. R. Weldon, started his career at about the same time (1883) with embryological work, his first contribution being a note on the early development of Lacerta muralis in vol. 23. Both he and Bateson forsook embryology for other fields of zoological research, and took divergent paths, but it is of interest to record that their earliest studies were typical of the Cambridge school as influenced and directed by Balfour.

Somewhat earlier in point of time were the memoirs of another distinguished Cambridge embryologist, A. Milnes Marshall, who, like Balfour, lost his life in a mountain accident at a young age. His works on the development of the cranial nerves of the chick and on the head cavities and associated nerves of Elasmobranchs are to be found in vols. 18 and 21 of the Journal.

The modern study of mammalian development in England may be said to have been inaugurated by another Cambridge embryologist, W. Heape, whose papers on the development of the mole appear in vols. 23, 26 and 27. To him succeeded A. A. W. Hubrecht, of Utrecht, with a series of studies on the embryology of the hedgehog (vol. 30) and of the shrew (vols. 31 and 35), followed by his fine memoir on the fœtal membranes of the Tarsiidæ and Lemuridæ, entitled, "Spolia nemoris," in vol. 36 (1894). Shortly afterwards we come across the remarkable memoirs of J. P. Hill and J. T. Wilson on the placentation and embryology of the Marsupialia, in vols. 40, 43 and 56. R. Assheton is another notable contributor to mammalian embryology in a number of memoirs extending from vol. 37 (1894) to vol. 54 (1909). The papers of J. W. Jenkinson on the early stages of the development of the mouse (vol. 43) and on the placenta of a lemur (vol. 61) must not be left out of account in the history of this very special feature of the 'Quarterly Journal of Microscopical Science,' nor the important work of Willey on the placentation of the beaver (vol. 60, 1915), and of J. P. Hill, on the Early Development of Didelphys (vol. 63, 1918).

Other notable embryological memoirs are those of A. S.

Packard on the embryology of Limulus polyphemus in vol. 11 (1871); of C. O. Whitman on the embryology of Clepsine in vol. 18 (1878)—this is one of the earliest papers in which a cell-lineage is recorded; of J. Graham Kerr on the development of Lepidosiren paradoxa (vols. 45, 46 and 54), and of E. W. MacBride on the development of Echinoderms (vols. 34, 38, 42, 51, 58 and 59).

If we now turn to memoirs on descriptive and comparative anatomy the list becomes too long for enumeration, and only a small selection can be attempted. There are H. N. Moseley's descriptions of pelagic and land Planarians in vol. 17; his notes on the structure of Seriatopora, Pocillopora, Corallium and Tubipora in vol. 22, which formed the starting-point for subsequent work on the Anthozoa by S. J. Hickson, G. H. Fowler and G. C. Bourne (vols. 23 to 28); and specially Moseley's last and very remarkable discovery of the presence of eyes in the shells of certain Chitonidæ (vol. 25, 1885). There is a fine set of ten papers on Echinoderm morphology by P. H. Carpenter, extending from vol. 18 (1878) to vol. 28 (1888). W. Baldwin Spencer's memoir on the presence and structure of the pineal eye in Lacertilia (vol. 27, 1887) was a most striking contribution to science, and the interest in the subject has been fully maintained by A. Dendy in vols. 42 and 51. E. B. Poulton's discovery of the true teeth underlying the horny plates of Ornithorhynchus is announced in vol. 29. The monographic works of A. Gibbs Bourne, F. E. Beddard and W. B. Benham on Oligochæta and leeches have already been mentioned. The interesting papers on Nautilus, Ctenoplana, Heteroplana, Ptychodera, and other novelties, sent home from the South Pacific by Lankester's former pupil and assistant, Arthur Willey (vols. 39 and 40) deserve especial mention. The Porifera have been elucidated in great detail by E. A. Minchin, especially in his "Materials for a Monograph of the Ascons," in vols. 40 and 52, and by A. Dendy in vols. 29, 32, 35, 36 and 60, and by Woodland in vols. 49 and 52. Also we must cite the work of R. Evans, of Jesus College, Oxford, on Spongilla, vols. 41, 42, and 44.

A very instructive piece of work carried out under the advice of Sir Ray Lankester is that of E. S. Goodrich on the fossil Mammalia of the Stonesfield Slate (vol. 35).

In the domain of Protozoology the Journal is very strongly represented. W. Archer, for many years a co-editor, was a renowned microscopist, and the earlier volumes are enriched by his numerous descriptions of Diatoms, unicellular Algæ and Heliozoa. Perhaps his most interesting memoir is that on Chlamydomyxa labyrinthuloides, published in vol. 15 (1875). A description of an allied species, C. montana, is given by Sir Ray Lankester in vol. 39. Among names that are famous we may note that of Otto Bütschli, whose researches on the Flagellate Infusoria and allied organisms are published in vol. 19 (1879). In the same year and volume T. R. Lewis made known his researches on flagellated organisms in the blood of healthy rats and his discovery of the Nematoid Hæmatozoa of man, and there follows a memoir in vol. 24 on further observations on flagellated organisms in the blood of animals. Little more is heard of these organisms for many years, but in vol. 45 (1902) the subject is revived by J. R. Bradford and H. G. Plimmer's paper on Trypanosoma Brucii, the organism found in Nagana, or tsetse-fly disease, and in vol. 49 trypanosomes are again recorded, along with other organisms, in Castellani and Willey's observations on Hæmatozoa in Ceylon. After this we get H. M. Woodcock's lengthy review of the Hæmoflagellates in vol. 50, followed by his studies on Avian hæmoprotozoa in vols. 50, 58 and 60, and his notes on Sporozoa in vol. 58. Parallel with these are the papers by E. A. Minchin in vols. 52 and 53, by Minchin and Woodcock in vols. 55 and 57, and Minchin and J. D. Thomson in vol. 60. The recent volumes contain a large output of protozoological work, and the names of Clifford Dobell, Muriel Robinson, Annie Porter, H. Pixell Goodrich and C. H. Martin are deserving of special mention among more recent authors.

Protozoology at certain points shades almost imperceptibly into pathology, and the Journal is not deficient in contributions to the latter subject. In early days there are memoirs by Lord Lister; the last, in vol. 21, is on the relations of micro-organisms to disease. Vol. 24 is enriched by an article by E. Metschnikoff on the ancestral history of the inflammatory processes.

As is befitting in a journal devoted to microscopical science, a large amount of space, especially in the earlier volumes, is devoted to histology. In this department E. Klein, a co-operator in the editorship from 1878 to 1892, stands pre-eminent with twenty-eight memoirs, the last from his pen appearing in vol. 36, when several of Sir Ray Lankester's friends and associates contributed special papers in commemoration of the twenty-fifth year of his editorship. The majority of Klein's papers deal with the normal histology of the tissues, but not a few relate to the histology of pathological conditions, and two at least on the structure of cells and nuclei, in vols. 18 and 19, cross over the indefinite borderline that separates histology from its more modern development, cytology. There are two definitely histological papers by E. A. Schäfer in vols. 15 and 18, but it is an impossible task to fix upon this or that paper among the many that have been published in the last fifty years and say that this or that memoir is mainly histological. A great proportion of them are studies in minute anatomy, involving faithful descriptions of the tissues of animals and plants, and if one should ask where are the histological memoirs in the Journal, the answer is passim: there are but few in which histology does not enter to a considerable extent. But there are some which afford definite landmarks in the course of cytological research, and among the most prominent of these are that by J. B. Farmer and J. E. S. Moore on the Meiotic phase in animals and plants (vol. 48), C. C. Dobell's review and criticism of Chromidia and the bi-nuclearity hypothesis (vol. 53), C. F. U. Meek's 'Problem of Mitosis' in vol. 58, R. R. Gates and N. Thomas's cytological study of species of Œnothera in relation to mutation (vol. 59), J. O. W. Barratt and G. Arnold's memoir on changes in Chondriosomes occurring in pathological conditions (vol. 58), and lastly the remarkable memoirs of J. B. Gatenby in vols. 62 and 63 on Mitochondria and the Golgi apparatus in the cells of various animals. Of like import are the careful memoirs of L. Doncaster in vols. 49, 51 and 58, in which the methods are cytological, but the aim is the elucidation of certain problems in gametogenesis. He sums up the existing state of our knowledge in a masterly essay on chromosomes, heredity and sex in vol. 59.

In recent years the tendency of zoologists has been to make increased use of the experimental method in research—a tendency which is not so much a new departure as a return to the practice of older days when animal physiology was not yet divorced from morphology. There has also been a parallel movement towards the practice of older days in the increased attention given to the intensive study of the natural history of animals, or, as it is now called, "bionomics," a term first introduced by Ray Lankester in his article "Zoology" in the 'Encyclopædia Brittanica,' 1889. These in many ways closely associated tendencies are reflected in the pages of the Journal. There are the altogether admirable researches of B. Grassi and A. Sandias on the constitution and development of the society of Termites in vols. 39 and 40; the researches of F. W. Gamble and F. W. Keeble on Hippolyte varians in vol. 43 and on the Bionomics of Convoluta roscoffensis in vol. 47; W. Garstang's paper on modifications of structure subservient to respiration in Decapod Crustacea in vol. 40; Geoffrey W. Smith's series of eleven studies in the experimental analysis of sex, extending from vol. 54 to vol. 60; E. W. MacBride's studies on the crossing of Echinocardium cordatum with Echinus esculentus in vol. 58; C. Shearer's work on Echinoid hybrids, in conjunction with W. de Morgan and H. M. Fuchs in vol. 58, and in conjunction with D. J. Lloyd on methods of producing artificial parthenogenesis in Echinus esculentus and rearing the resulting plutei through metamorphosis, in the same volume. Others might be cited, but it is not the object of this article to give a complete list and mete out equal justice to all contributors. The intention is rather to pick out in a somewhat random way such memoirs as serve to illustrate the character of the 'Quarterly Journal of Microscopical Science' during the last fifty years, and to illustrate the manner in which it has kept pace with and often set the pace to the various developments of biological activity that have manifested themselves during that period. Authors whose names have been omitted will excuse the omissions when they consider the impossibility of doing justice to all claims for recognition of good work within the limits of a short article.

This review, however, would be incomplete if it did not make mention of two very important auxiliaries to the success and usefulness of the Journal. Copious and accurate illustration is essential in descriptive zoology, and from its foundation the 'Quarterly Journal' has been generous as regards the number, insistent as regards the quality of the lithographic plates illustrating the memoirs. Mr. Tuffen West was the first and for many years remained the lithographer to the Journal. Occasionally the execution of the plates has been entrusted to foreign firms, such as MM. Severeyns of Brussels and Werner and Winter of Frankfort. But for many years nearly the whole of the lithographic work has been in the hands of Mr. Seton Huth, of Cricklewood. His father's name first appears on the beautifully executed plates illustrating the memoirs of Mr. William Archer on Freshwater Rhizopoda and of Sir Ray Lankester "On the Coincidence of the Anus and Blastopore in Paludina," in vol. 12 (1876). Mr. Seton Huth has rendered devoted service to the Journal, and his personal interest in its welfare has been conspicuous during the last four years, when the exigencies of war created difficulties that seemed almost insuperable. But they were overcome successfully, and if a falling off in the quality of the paper was unavoidable the execution of the drawings shows no deterioration.

Another most important auxiliary is a reliable index. The first, published in 1890 as part of No. 117, vol. 30, was compiled by Dr. G. H. Fowler, and includes the old series and the transactions of the Microscopical Society up to 1869. The second, published in 1916 as part of No. 245, vol. 62, is the work of Dr. Helen Pixell Goodrich, who followed closely the method adopted by her predecessor. These two indices, admirable in arrangement and accuracy, are of the greatest use to all readers of the Journal.

The mere record of half a century's effort and output, while bearing ample witness to Sir Ray Lankester's energy and versatility, would of itself fail as a recognition of his services to British zoologists if account were not also taken of the genial personal relations established with them through the medium of his editorship. To each successive generation of aspirants to the honour of having their early and often immature work published in the 'Quarterly Journal of Microscopical Science' he has been a guide, philosopher and friend, appreciative, fruitful in suggestion, and full of encouragement towards further efforts. As he has given freely from his large store of judgment and experience, so he has received in return the esteem and confidence of an increasing circle of friends, whose intimacy with him and with one another has contributed largely to the solidarity of British zoologists. On this fiftieth anniversary they unite in offering him their cordial congratulations on the achievement of past years coupled with their best wishes for those that are to come.

The Pseudopodia of the Leucocytes of Invertebrates.

By

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With Plates 1 and 2.

It is to Wharton Jones that we owe the first comprehensive account of the leucocytes in the various groups of Invertebrata: in an important paper, published in 1846 (**6**), he described and figured them as of "stellate appearance," and sometimes as shooting out "cilia-like processes." Since then numberless authors have studied these cells and pictured them as provided with free outstanding pseudopodia. Evidence is brought forward in this paper that the spiny appearance of leucocytes is of the nature of an optical illusion or due to changes taking place under abnormal conditions, that freely projecting fine processes are seldom, if ever, produced in the fluids of living invertebrates, and that the pointed pseudopodia so often figured are merely the optical sections of delicate, more or less folded films.

Various authors have from time to time mentioned the flattened expanded shape of the processes of the leucocytes of the invertebrates, as, for instance, Cattaneo (**1**) and Dekhuysen (**2**), referred to below; but none seem to have realised that this is the normal shape of the pseudopodia. In 1898 (**4**) I drew attention to the presence of membranous expansions of the leucocytes in the Polychæte worm Glycera, and at that time observed the same structures in other invertebrates; but other work prevented my pursuing the subject, and it was not till last winter that it was again taken up, when I had the opportunity of confirming and extending my observations at the Marine Biological Laboratory in Plymouth.

No attempt will here be made to describe in detail the various kinds of corpuscles found in the blood or cœlomic fluid, nor will the present paper deal with their granular contents. These subjects have been dealt with by many writers, more especially by Cuénot and by Kollman, who gives a very complete bibliography in his excellent memoir (**8**).

As examples of the Annelida we may take the Polychæte Arenicola and the Oligochæte Lumbricus. The cœlomic fluid of Arenicola contains abundant rounded and oval corpuscles, with others of intermediate form. At first sight the rounded cells seem to be provided with numerous pointed processes projecting in all directions; but more careful examination under the oil-immersion lens reveals the fact that these "pseudopodia" are in reality but optical sections of membranous expansions of cytoplasm thinning out peripherally to a very delicate and almost invisible film (Pl. 1, figs. 2 A and B). Here and there the membrane is strengthened by a rib, which appears as an outstanding process under a lower power.

The cells floating in the cœlomic fluid of Lumbricus, so often figured as provided with fine, frequently branching processes (Metchnikoff [**9**], Keng [**7**]), can, if examined quite fresh, be seen to throw out very delicate membranes. These, being somewhat folded and crinkled, give the appearance in optical section of long, branching pseudopodia (Pl. 1, figs. 3 A, B, C). The membrane of the leucocyte of the earthworm is thinner than that of Arenicola, hence the processes seem to be more delicate.

It is difficult to decide whether several independent membranes are produced, or whether the various lobes often seen passing out from the cell in different planes are really formed by the complicated folding of a single sheet of cytoplasm extending originally all round its edge. In Arenicola it is usually of the latter structure (Pl. 1, figs. 1 A, B), and a favourite attitude is that shown in Pl. 1, fig. 2 A, where the cell and its membrane somewhat resemble a half-opened flower. On the other hand, in the earthworm the membrane in freely floating cells is much folded and seems to spring from any point on the surface (Pl. 1, fig. 3 B). Frequently in Lumbricus, as well as in Arenicola, there appear to be several separate outgrowths.

Very similar to the leucocytes of the Annelids are the cells floating in the blood of the Mollusca. In Pl. 1, figs. 5, 6 and 7 of the leucocytes of Ostrea and Mytilus show the extensive membranes, the thickenings and folds of which yield the deceptive appearance of delicate pseudopodia.

As an example of the Arthropoda we may take the shore crab, Carcinus mænas. Its blood contains rounded, granular corpuscles, which may produce a large process at one side only (Pl. 1, fig. 9 B), or various processes projecting in all directions and thinning out to delicate films bearing many sharp points (Pl. 1, fig. 9 A).

The Echinoderms have long been known to possess very "amœboid" cœlomic corpuscles. In his interesting paper on "plasmodium" formation (3) Geddes figures the leucocytes of Echinus as provided with very long, branching pseudopodia, some of which join so as to encircle a space. In reality no such processes are present in fresh corpuscles, and they, like the ring-like arches, are merely representations of optical sections of an extensive membrane folded with rounded surfaces (Pl. 2, fig. 11). Very soon, however, after the cœlomic fluid has been removed from the animal the membrane begins to stretch out wing-like films in various directions, and these soon acquire a jagged edge with sharp points (Pl. 2, fig. 12).

In the above brief review have been selected from the various groups only a few typical examples out of the large number of genera examined. They are sufficient to show that the formation of delicate lamellæ of cytoplasm is characteristic of the leucocytes of all the invertebrate Cœlomata. When the cells are freely floating in a hanging drop the membranes are seen to be motile, continually undergoing change of shape, and capable of being more or less completely withdrawn. This motility varies considerably: in some cases they can be observed to wave about quite quickly, while in others they seem almost still. Whenever they come into contact with a foreign object, as, for instance, a glass slide or a cover-slip, the membranes tend to cling to it and spread over its surface as a thin film, the edge of which extends like a drop of oil on water. This well-known habit, observed by Metchnikoff (**9**) and many others, is doubtless related to changes of surface tension, and is of great importance to the animal in dealing with intrusive foreign particles and parasites entering the body-fluids. Though marked in Annelids and Molluscs (Pl. 1, fig. 5), it is still more pronounced in Crustaceans and Echinoderms, leading most of the cells to flatten themselves as a mere film over a glass surface. As shown by Theel (**12**), a piece of cover-slip introduced into a living starfish will very soon become almost completely covered and can then be conveniently stained and mounted (Pl. 2, fig. 14). Tait has recently suggested the name "thigmocyte" for these cells in the Crustacea (**11**).

Moreover, it has long been known that the body-fluids of the Invertebrata rarely clot (as in the Vertebrata and some Crustacea) by fibrin formation, but more usually by the gathering together of the leucocytes in irregular clumps and strands. These are the so-called "plasmodia" first described in detail in Echinus by Geddes (**3**), who thought the leucocytes became completely fused and formed a mass comparable to the plasmodia of Myxomycetes. But I agree with Michel (**10**) that the cells do not really lose their identity, are merely connected by their hyaline ectoplasmic layer, and are capable of resuming their independence under certain conditions. That this very marked tendency to form such agglutinations is due to the presence of the membranous extensions described above there can be little doubt, and that it is a most useful property is due to the fact that it serves not only to close wounds, but also to surround foreign particles or parasitic organisms too large to be dealt with by a single leucocyte (**3**, **5**).

It now remains to be proved, firstly that the membranous pseudopodia are not artefacts, are not due to contact with foreign bodies, and are present in the expanded condition on cells floating in the fluids of the living animal; and secondly, that the delicate radiating pseudopodia sometimes seen are not normally developed in these fluids, and are only formed under abnormal conditions. For this purpose a technique must be adopted which will enable us to fix the leucocytes rapidly in their normal state, without shrinking the membranes beyond recognition. Having tested many of the well-known fixatives, such as osmic acid, Flemming's fluid, Bouin's fluid, corrosive acetic, etc., I find the only reagent giving a satisfactory result is a watery solution of iodine. A strong solution of iodine in potassium iodide may be used, diluted to about the colour of sherry with normal saline for terrestrial and freshwater animals, and with sea-water for marine forms. If desired it can be followed by Bouin's fluid, and the cells stained and mounted in balsam. Such a solution of iodine introduced under the cover-slip fixes the finest pseudopodium or the most delicate protoplasmic film with marvellous rapidity and precision; and fluid dropped straight into the solution from the animal displays the leucocytes fixed in their natural condition.

Now if this test be applied to the cœlomic fluid of Arenicola the vast majority of the leucocytes are fixed with a wide sheet of protoplasm extending all round, and giving them somewhat the appearance of an open umbrella, as shown in Pl. 1, figs. 1 A and B. Although most of the rounded cells are of this type, there are many with more or less pronounced ridges or extended films projecting from the cell-body in various directions (Pl. 1, fig. 1 C). Again, in the earthworm the leucocytes are likewise found fixed with membranous extensions irregularly lobed and sometimes of relatively enormous size (Pl. 1, figs. 4 A and B). Similarly in the blood of Molluscs the membranes are seen to be more or less completely extended (Pl. 1, fig. 6). Most striking of all, perhaps, is the case of the Echinoderm. In Asterias, for example, all the leucocytes display a much-folded membrane, bulging in a characteristic way so as to give them a vesicular appearance (Pl. 2, figs. 11 A–D); but so far as I have been able to make out the vesicles are not really closed, but always have an opening bounded by the thin free edge of the membrane. On the other hand, the blood of Crustacea dropped into iodine solution seems to show that in them the membranes are not so much expanded; for although often quite recognisable in spite of the abundant coagulum they are generally short, and may be represented merely by blunt pseudopodia—at all events in the species I have studied (Carcinus mænas, Eupagurus prideauxii, Astacus fluviatilis).

It is quite clear, then, that in most invertebrates the membranous pseudopodia of the leucocytes are normally expanded in the living animal, and it is equally clear that these cells are not provided with fine outstanding pseudopodia. Although not prepared to assert that such processes never exist in the normal body-fluids, I can state that they were not found in preparations of freshly-drawn material. Nevertheless they undoubtedly rapidly make an appearance in blood or cœlomic fluid allowed to stand, and may often be of extraordinary length and tenuity (Pl. 2, figs. 13 A, B). Leucocytes floating in a hanging drop may thus come to resemble delicate Heliozoa with fine processes radiating all round them. The formation of such fine pseudopodia is almost certainly abnormal, and due to physico-chemical changes taking place in the fluid, and possibly a sign of approaching death. As to their mode of origin, it seems probable that they are not spun out as free-growing threads, but are rather the remnants of previously existing membranes, being the thicker ridges or supporting folds which remain when the intervening film has thinned out or retracted. The appearance of certain "thigmocytes," such as that shown in Pl. 1, fig. 8, strongly suggests this interpretation, and the dentated edge of the membranous folds so frequently seen (figs. 9 A and 12) may represent initial stages in the production of pointed pseudopodia. The leucocytes of Arenicola figured by Dekhuysen appear to be in this condition (**2**).

The chief results recorded in this paper may be briefly summarised as follows: The leucocytes of the blood or cœlomic fluid of the invertebrate Cœlomata are provided with more or less extensive membranous processes of cytoplasm. The freely-projecting pseudopodia usually described are either figured from optical sections of the folded membranes or from cells which have produced them under abnormal conditions. These fine pseudopodia may be present on cells in fluid withdrawn from the body and which has been allowed to stand, and are probably derived from pre-existing membranes. The delicate motile membranous folds are usually expanded in the normal fluids of the living animal.

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EXPLANATION OF PLATES 1 and 2,

Illustrating Mr. Edwin S. Goodrich's paper on "The Pseudopodia of the Leucocytes of Invertebrates."

[All the figures, except fig. 2, have been drawn with the camera lucida at a magnification of 1600 diameters.]

PLATE 1.

Figs. 1 A, B and C.—Three cœlomic corpuscles of Arenicola grubei, fixed in iodine. B, in profile; C shows membranes projecting in several planes.

Figs. 2 A and B.—Cœlomic corpuscles of Arenicola ecaudata, drawn from the living. × 1600.

Figs. 3 A, B and C.—Cœlomic corpuscles of Lumbricus herculæus, drawn from the living.

Figs. 4 A and B.—Two cœlomic corpuscles of Lumbricus, fixed in iodine.

Fig. 5.—Blood-corpuscle of Ostrea edulis, much flattened on a cover-slip; from the living.

Fig. 6.—Blood-corpuscle of Ostrea edulis, fixed in iodine.

Fig. 7.—Blood-corpuscle of Mytilus edulis, from the living.

Fig. 8.—Blood-corpuscle of Carcinus mænas, much flattened on a cover-slip and fixed in iodine.

Figs. 9 A and B.—Blood-corpuscles of Carcinus mænas, from the living. A shows two corpuscles agglutinated and floating; B, a single corpuscle adhering to glass.

PLATE 2.

Fig. 10.—Cœlomic corpuscle of Asterias glacialis, fixed in iodine.

Figs. 11 A, B, C and D.—Cœlomic corpuscles of Asterias glacialis, fixed in iodine and Bouin's fluid, stained, and mounted in balsam.

Fig. 12.—Cœlomic corpuscle of Asterias glacialis in a hanging drop; from the living.

Figs. 13 A and B.—Cœlomic corpuscles of Asterias glacialis, flattened out on a slide; drawn from the living.

Fig. 14.—Two agglutinated cœlomic corpuscles drawn from a cover-slip inserted into an Asterias glacialis and fixed with iodine.

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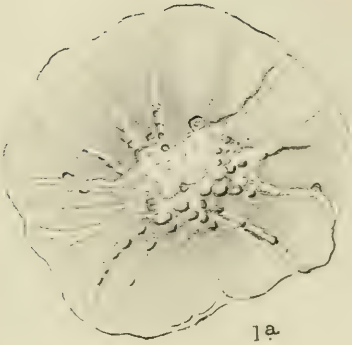

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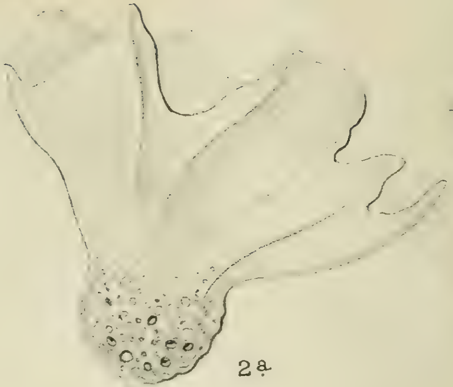

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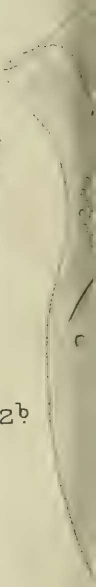

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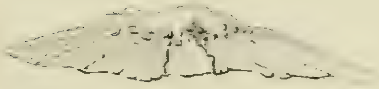

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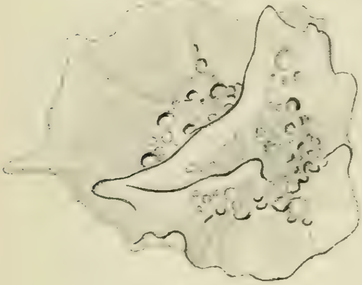

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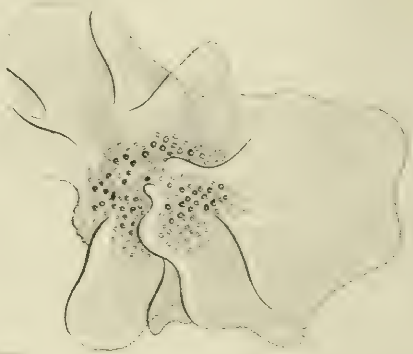

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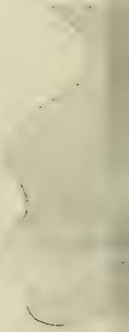

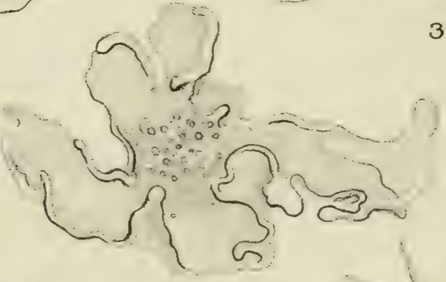

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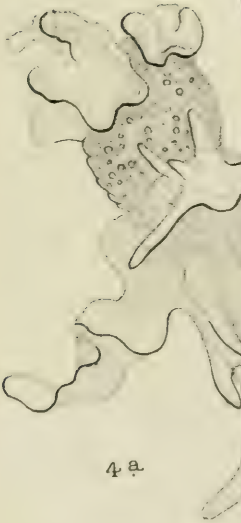

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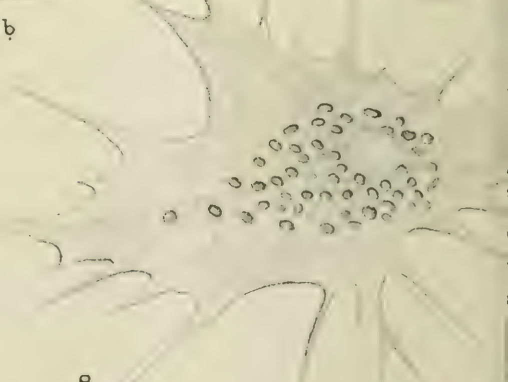
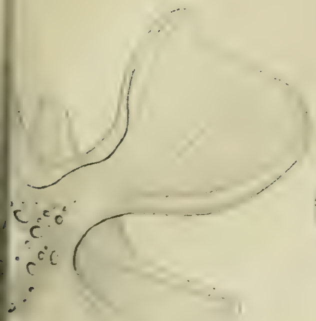
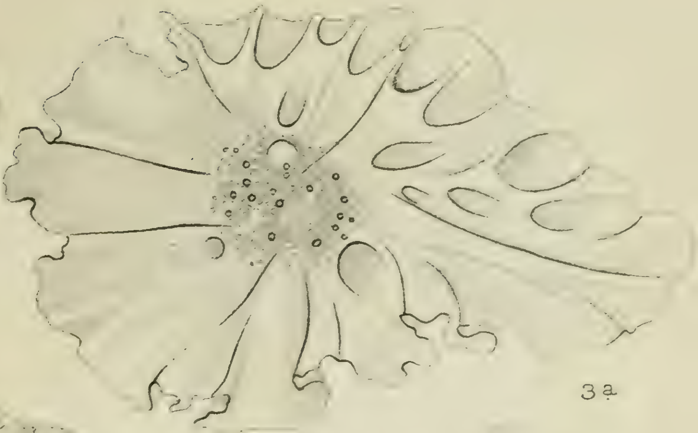


8.

E.S.Goodrich del.

LEUCOCYTE





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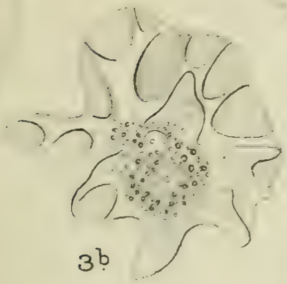

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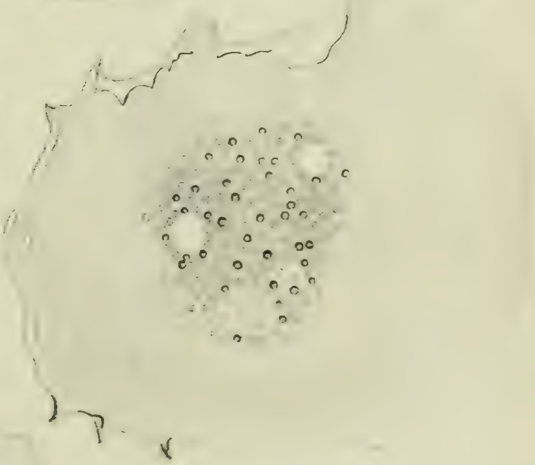

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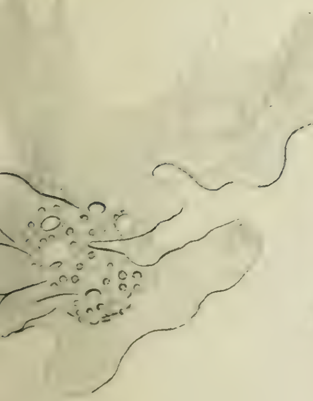

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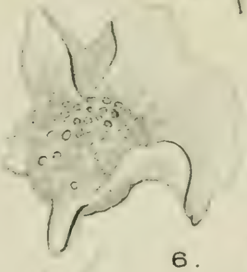

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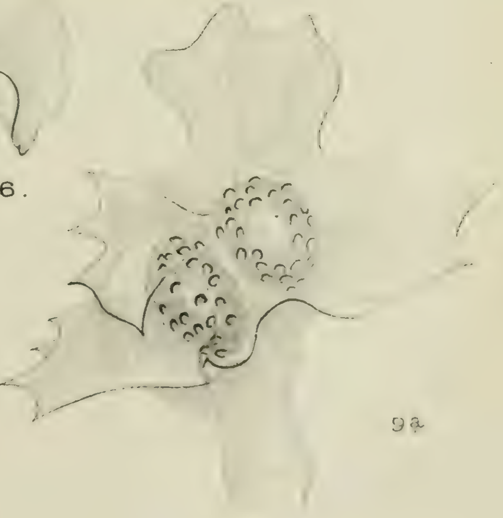

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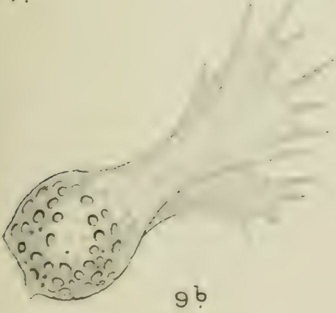

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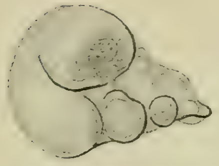
NVERTEBRATA.

Quart. Journ. Micr. Sci. Vol. 64, N.S. Pl. 2.

26B



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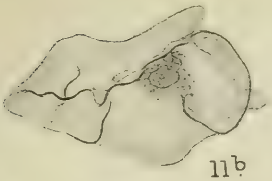

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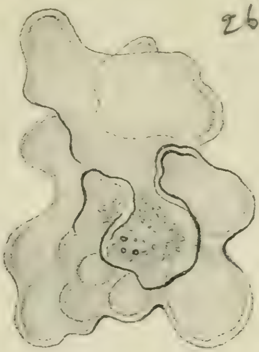

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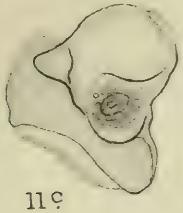

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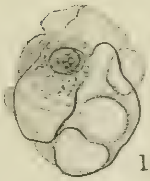

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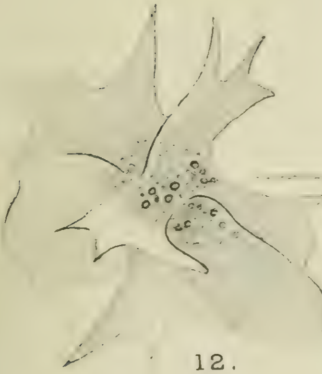

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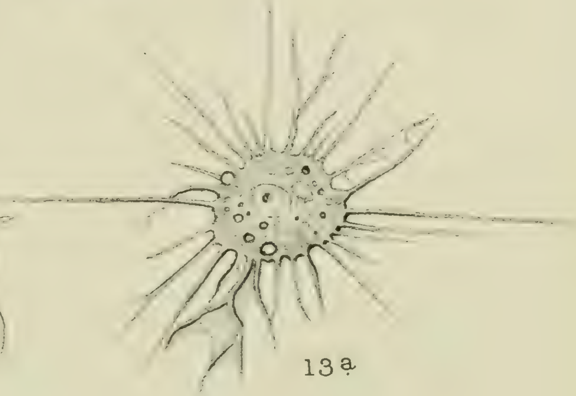

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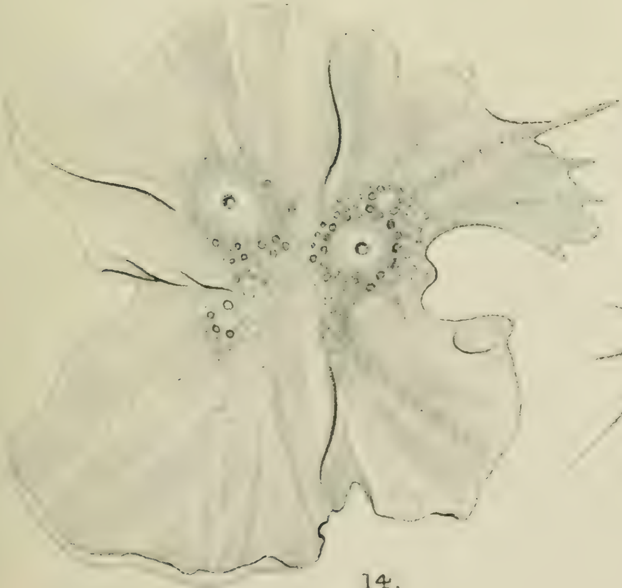

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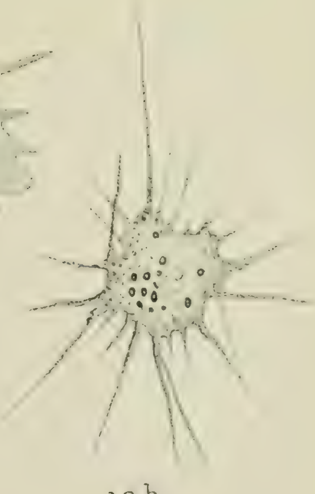

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LEUCOCYTES OF INVERTEBRATA.

Observations on Arachnactis albida, M. Sars.

By

Gilbert C. Bourne, M.A., D.Sc., F.R.S.,

Linacre Professor of Zoology and Comparative Anatomy and Fellow of Merton College, Oxford.

With Plates 3, 4, and 5.

By the courtesy of Mr. T. A. Stephenson, of the University College of Wales, Aberystwyth, I have been entrusted with a portion of the collection of Anthozoa made by the Department of Agriculture and Technical Instruction for Ireland, in the course of marine investigations extending over a number of years. Among these were several gatherings of Arachnactis larvæ, and I have welcomed the opportunity of resuming studies which I began, but never brought to a conclusion, in 1889. At that time little was known of the anatomy and life-history of the Arachnactis larvæ of Cerianthus beyond what was contained in the works of M. Sars (**20**) and A. Agassiz (**1** and **2**), and finding that a species, subsequently named Arachnactis bournei by Fowler (**13**), was a constant element in the plankton collected at Plymouth in the summer months, I selected and preserved for microscopical study as many stages as possible, intending to publish a full account of my investigations of their structure. But hardly was the material for my projected work collected when I was asked to allow the late Prof. E. van Beneden to look at it. I accordingly sent all the specimens of Arachnactis that I possessed to the distinguished Belgian zoologist, without attaching any conditions to what I regarded as a loan of valuable material. In the absence of such conditions the loan was taken to be a gift. Prof. van Beneden made prompt use of the material that I had provided, and within a year had written the memoir (**3**) which has to a large extent formed the basis of his own and other subsequent work on the subject. I have no complaint to make against an esteemed and lamented friend. Prof. van Beneden gave me more than my due share of credit in the matter, and doubtless made fuller and better use of the material than I should have done at the time. I only mention the matter now because a survey of the literature, in which my name occurs pretty frequently, suggests that I acted as a mere collector of material and threw away a good opportunity through failure to appreciate the value of what I had preserved. As it happened, a subject which I had hoped to make my own passed into other and very able hands, and the collections that I made were the prelude to the splendid memoir afterwards published by Prof. E. van Beneden (**4**) on the Anthozoan larvæ of the "National" Expedition. More recently large additions to our knowledge have been made by Fowler, Carlgren, McMurrich and others, and the developmental history of at least two species of Arachnactis has been so fully treated by them that little seems to be left for any subsequent investigator to deal with. But the Irish collections that have come into my hands enable me to fill some gaps in our knowledge of the life-history of one species—A. albida.

The specimens of Arachnactis collected by the Fisheries Branch of the Department of Agriculture for Ireland may be divided into two groups.

I. Those from collections made in the Irish Sea in 1910, from two localities.

Ia. May 3rd, 1910; 17 miles E. ½ S. of Rockabill. N. lat. 53° 39′ 30″; W. long. 5° 32′. Coarse silk and cheesecloth tow-net at 30 fath. This haul gave fifty-four examples of Arachnactis, all at approximately the same stage of development, with 9–11 marginal tentacles. The label bears the name Arachnactis bournei—a synonym of A. lloydii. The specimens collected by me at Plymouth in 1889 were described by van Beneden (**3**) as the "larve de Bourne," but he did not at first give them a specific name. Subsequently (**4**) he identified them as the larval forms of Cerianthus lloydii, but Fowler (**14**) has pointed out that this identification is conjectural, and proposed the specific name bournei for this larva. These examples from the Irish Sea bear a close resemblance to others of a slightly younger age which I obtained at Plymouth in 1914.

Ib. Five examples, similar to the above, taken May 12th, 1910, 14 miles S. of Hook Light. N. lat. 51° 54′; W. long. 6° 40′. Coarse silk and cheesecloth tow-net at 18 fath. Soundings, 38 fath.

I have given in Pl. 3, figs. 1 and 2, outline drawings of one of these larvæ, to illustrate the marked difference in form between them and the corresponding stages of Arachnactis albida, about to be described. The specimen figured has eleven marginal tentacles, the anterior median tentacle well developed but shorter than those of the adjacent first couple. The first, second and third couples of tentacles are fully formed, the fourth couple much smaller, and the fifth couple represented by mere nodular swellings. There are only two couples of labial tentacles, with rudiments of a third couple. These figures should be compared with Pl. 3, figs. 12 and 13, representing equivalent growth stages of Arachnactis albida. In the latter there are three couples of labial tentacles, and in Pl. 3, fig. 13, rudiments of a fourth couple.

According to van Beneden, A. lloydii (= A. bournei Fowler) is distinguished from A. albida by three sets of characters, well shown in Pl. 3, figs. 1 and 2. (1) The relatively small size and shortness of the tentacles, which are pointed-digitiform and without the terminal pigmental swellings characteristic of albida. (2) By the ovoid and even cylindrical shape of the body. (3) By the relatively early appearance of the anterior median marginal tentacle in

A. bournei, which, he says, coincides in time of appearance with the fourth couple of mesenteries. I have, however, a drawing of a specimen of A. bournei, taken at Plymouth in 1914, in which the anterior median marginal tentacle is distinctly represented by a small outgrowth, but only three couples of mesenteries are present, and, as I shall show in the sequel, the time of appearance of the median marginal tentacle is subject to much variation in A. albida. I must add as a fourth distinction, also noted by McMurrich, that the labial tentacles appear earlier and are always more numerous relatively to the number of marginal tentacles present at any stage in A. albida than in A. bournei. Thus in Pl. 3, fig. 3, which is a careful drawing of the youngest stage of A. albida in the Irish collections, and is indeed the youngest yet figured or described, there are distinct indications of the presence of two couples of labial tentacles. In my drawings of corresponding and even later stages of A. bournei from Plymouth there is no trace whatever of labial tentacles.

Beyond these remarks I have nothing to add to our knowledge of A. bournei.

II. The second group comprises three different gatherings made in approximately the same locality, from deep water on the bank stretching out into the Atlantic west of Bantry Bay.

IIa. From Station 31 on August 7th, 1903, fifty miles W.N.W. of Tearaght; Garstang net at 40 fath. Soundings, 306 fath. This sample comprises several hundreds of examples of Arachnactis albida in various stages of development, and has afforded the principal material for this memoir. Unfortunately the catch was not too well preserved, and came into my possession in a screw-stoppered jar, from which spirit readily evaporates; so the histological condition, especially of the delicate younger forms, leaves much to be desired. Some of the older forms, however, are in a tolerably good state of preservation.

IIb. Station 1237, 69 miles W. by N. of Tearaght Light. N. lat. 51° 54′; W. long. 12° 29′. Midwater otter meso-plankton trawl at about 450 fath. Soundings 569 fath.

This gathering comprises six specimens of Arachnactis, of which the largest, obviously much contracted by reagents, measures 4·5 mm. in length × 3 mm. greatest diameter. They correspond very closely to the oldest larvæ figured by Boveri (**5**, Taf. xxi, figs. 5^a, 5^b, 5^c) and, like his specimens, have lost the marginal tentacles almost entirely, but in one of the Irish specimens the attenuated remains of two of the larger marginal tentacles are still attached, and these measure 6·5 mm. from the edge of the oral disc to the tip. These larvæ are opaque; the general colour of the body in spirit is pink with diffuse dark brown pigmentation in the middle zone.

IIc. Station 1242, August 14th, 1911, 56 miles W. $\frac{1}{4}$ S. of Great Skellig. N. lat 51° 27′; W. long. 11° 51′. Mosquito-net and cheesecloth tow-net on trawl. Soundings, 550–590 fath.

Presumably the trawl was dragged along the bottom at this depth, and the tow-net attached to it was set to catch organisms swimming near the bottom. The catch includes eighteen examples of Arachnactis of various sizes and ages, but all with more than five couples of marginal tentacles. They are obviously identical with the forms taken in catch IIb, and, like them, have in most cases lost the marginal tentacles, a ring of perforations marking the places to which the tentacles were attached. The histology of these specimens was satisfactory. They are undoubtedly Arachnactis albida, M. Sars.

Much has already been written on Arachnactis albida, but as it is of comparatively rare occurrence and inhabits seas not continuously searched by naturalists our information in respect of it still remains imperfect. The most recent account is that of Carlgren (**9**), which follows an excellent summary of our knowledge of the species published six years earlier (**7**). In the latter paper he gives the following definition of the species (translated): Marginal tentacles long, in earlier developmental stages many times longer than the distance between the oral and aboral ends of the body.

Body discoidal in the younger stages, subsequently more cylindrical with the aboral end rounded off; finally this part becomes pointed. The directive tentacle of the marginal tentacles first appears after the four first marginal tentacle-couples have attained a considerable length, and after three couples of labial tentacles and six couples of mesenteries have been formed. Mesenteries long, in older larvæ reaching nearly to the aboral end; in younger forms the part lying below the actinopharynx (Schlundrohres) is from half to one-third the length of the body. No food-yolk in the larvæ. The aboral pore is formed late (in the stage with twelve labial tentacles [Fowler] or with seventeen tentacles [Boveri]). Colour: Extremities of the marginal tentacles, the actinopharynx and filaments, brown. Labial tentacles coffee-brown. The remaining parts of the body colourless, transparent. Size: Large form—length of marginal tentacles from 35 to 40 mm. Length of labial tentacles 3–4 mm. Length of body up to 15 mm. Diameter of body up to 7 mm. (in preserved condition).

This diagnosis holds good for the numerous stages that I have been able to examine, with this exception, that I find the time of appearance of the directive marginal tentacle subject to much variation. In some specimens it is distinctly growing out when only two couples of labial tentacles and five couples of mesenteries are present. In other specimens again its appearance is deferred until seven couples of mesenteries are present.

The localities and depths at which the Irish collections were made deserve some attention. Carlgren (**7**) has given a full list of the localities from which A. albida has been taken up to 1912. Plotting them out on a map, it is obvious that the centre of distribution of this species is north of 57° N. lat., and lies between the Faroe Islands and the Scotch and Norwegian coasts. One catch is reported from as far North as 75° N. lat. and as far east as 32° 18′ E. long. Two catches have been made as far west as 20° W. long. But the few specimens taken by E. T. Browne (**6**) in Valentia harbour in 1895 and 1897 and all the specimens taken by the Fisheries Branch of the Department of Agriculture for Ireland come from a restricted area off the south-west coast of Ireland, near 51° N. lat., and thus constitute a more southern subcentre of distribution. From the fairly constant recurrence of the species in this more southern area (it has been taken there in 1895, 1897, 1903 and 1911), one may surmise that the adult form breeds in the neighbourhood.

In the great majority of cases the species has been found at or near the surface, and sometimes in swarms, as described by Vogt (**22**) and Vanhöffen (**21**). Fowler (**13**), who captured numerous examples in a series of hauls in the Faroe Channel, regarded albida as a purely epiplanktonic form, though he records as doubtful (as regards depth) specimens taken in 300, 400 and 480 faths. But the Danish Ingolf Expedition took specimens at 545 and 625 faths., and, as recorded above, two of the catches now in my possession came from 450 faths. and 550–590 faths. respectively. It is clear that A. albida, in its later stages at any rate, is mesoplanktonic.

As stated above, the catch of 1903 at a depth of 40 faths. contains an abundance of examples of A. albida in all stages of development. I have been able to make a series from an individual with four large and two smaller marginal tentacles and four couples of developed mesenteries up to the individual depicted in Pl. 3, fig. 17, with twelve marginal tentacles and twenty-one mesenteries. Though various authors have described many of the stages included in this series, and Fowler (**14**) has given a table which includes practically every stage except the oldest, the information is so scattered and the illustrations of the growth periods so incomplete that I propose to give drawings, with brief descriptions of the more important forms that I have studied, which, as I shall show, lead up nearly, if not quite, to the sexual form.

The youngest specimen of A. albida yet figured is that in Carlgren's memoir (**7**, p. 71, text figure 1^a). The youngest that I could find—and there was only a single example of this stage—is shown in Pl. 3, fig. 3. It is obviously at an earlier stage than Carlgren's specimen, having four well-developed and two smaller marginal tentacles; of the latter the right is, as usual, the larger. Two couples of short, blunt labial tentacles are clearly present. As shown in Pl. 3, fig. 4, the body is hemispherical, with the aboral end drawn out into a blunt projection.

Transverse sections (Pl. 3, figs. 5, 6 and 7) show that this larva has four couples of mesenteries attached to the actinopharynx, and in addition the rudiments of a fifth couple, represented by two minute ridges in the ventral chamber. The preservation being indifferent, it is not possible to make out histological details. Pl. 3, fig. 5, shows that there is already a differentiation between the epithelium of the sulcus and that of the rest of the actinopharynx. A thickening of both ectoderm and endoderm in the directive chamber marks the commencement of the formation of the directive marginal tentacle, but there is no outgrowth. Pl. 3, fig. 6, represents a section taken just below the level of the enterostome. The section has passed somewhat obliquely from in front posteriorly, so that the hyposulcus is not shown, but this structure is at this stage extremely short and hardly differentiated. The two directive mesenteries *d. d.* extend aborally for some little distance below the hyposulcus. They bear no filaments. The next three couples, viz. the second and third protomesenteries and the first couple of metamesenteries, bear filaments. The section portrayed in Pl. 3, fig. 7, passes through the body just below the bases of the tentacles, one of which, however, is included, and shows that the protomesenterial couples II and III and the first metamesenterial couple extend to a considerable distance aborally, but the directives have disappeared. The second couple is here much the largest and the filament is enlarged and contorted. At this stage there is no trace of an acontium on the first couple of metamesenteries, though, as the figure shows, there is a slight differentiation of the epithelium of their free edges. Calling this—the youngest albida larva that has yet been figured or described (unless Fowler's stage A, which he tabulates as having ten mesenteries, six marginal, and no oral tentacles is of the same age)—stage A, I will describe the older forms contained in the Irish collections as a series of stages B, C, D, etc., taking the number of the mesenteries as the basis of the series.

Stage B (Pl. 3, figs. 8 and 9) has eleven mesenteries—that is, five couples and one member of the sixth couple. There are three couples of long marginal tentacles and a couple of shorter tentacles, of which the right is the larger. The dorsal directive tentacle is represented by a small knob. There are two pairs of labial tentacles, of which the more dorsal couple opens into the third couple of actinocœles,[1] as was first pointed out by Boveri. This form is a little more advanced than Boveri's and a little younger than van Beneden's youngest specimen of A. albida.

Stage C.—Thirteen mesenteries, i. e. six couples plus the developing right-hand member of the seventh couple. Four couples of marginal tentacles, of which the left ventral is markedly shorter than the remainder. In the specimen depicted in Pl. 3, fig. 10, the dorsal directive tentacle is minute, smaller than in the specimen selected to illustrate stage B, but in other examples it is much larger, and nearly as long as the left ventral marginal. Three couples of labial tentacles, the second predominant in size. The body remains discoidal in this stage, and the second couple of mesenteries (counting from the dorsal end) are still predominant in size, and reach furthest down towards the aboral end. The ectodermal musculature of the body-wall is as yet feeble, though that of the tentacles is well developed. In some examples of this stage there are traces of a fourth couple of labial tentacles.

Stage D.—Seven couples of mesenteries, either complete, or the left member of the seventh couple rudimentary. Marginal tentacles nine or ten, viz. four couples of larger tentacles and generally a very small right member of the fifth couple, plus the median directive, which varies very much in development. It may be of considerable size, as in Pl. 3, fig. 12, but in other examples, such as that shown in Pl. 3, fig. 13, it may be so small as to be easily overlooked in a surface view. Three couples of labial tentacles always present, and in many examples more or less conspicuous rudiments of the fourth couple.

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[1] I coin this word as a convenient name for the intermesenterial chambers in a form which, as in Cerianthidæ, has no "exocœles" and "endocœles."

It is at this stage that important characters begin to make their appearance, leading from the larval to the sexually mature condition. The body still remains discoidal, even in such examples as that shown in Pl. 3, fig. 13, and the longitudinal musculature of the body-wall is still feeble in comparison with that of the tentacles. But the column is beginning to elongate, and it is at this stage that the fourth protomesenteric couple is first seen to surpass the second couple in length and to show traces of acontial outgrowths at the aboral ends. At this stage also a considerable advance is recognisable in the histological differentiation of the mesenteries and filaments. The fertile become quite distinguishable from the infertile mesenteries by the different characters of their filaments, as seen in transverse section. The endoderm at the sides of the mesenteries becomes much thicker, and begins to take on the form characteristic of later conditions and of the adult Cerianthid, and for the first time minute but distinct germ-cells may be recognised, forming clumps in close contact with the mesoglœal lamina of metamesenteries 1. Whilst most of the larvæ at this stage have the thin, elongated marginal tentacles which give the spider-like appearance from which the generic name of the larva is derived, a considerable proportion are found in the condition represented in Pl. 3, fig. 13, in which the tentacles are inflated and curved inwards over the oral disc, and the body is somewhat turgid. Examples in this condition almost always contain a bolus of food in the actinopharynx, the food almost invariably consisting of copepods, and this appears to be the stage at which the larvæ begin to feed freely on other relatively large-sized planktonic organisms. The increasing histological differentiation is doubtless correlated with the change in the habits of the larva, and the turgid condition of the full-fed forms is a phenomenon familiar to those who have kept and studied living Actinians. They generally become turgid after a meal.

No terminal pore is discoverable in any example of Stage D.

Stage E (Pl. 3, fig. 15).—Eight to 8½ couples of mesenteries. Marginal tentacles eleven or twelve, viz. one directive, now nearly equal in size to the largest of the remainder; four couples of larger and one couple or one and a-half couples of smaller tentacles at the posterior end. Labial tentacles, 4 couples, the last couple small.

The larvæ at this stage have increased considerably in size, and the majority of them are turgid and contain food. The body is now dome-shaped or ovoid and has increased in length. The acontia on metamesenteries 1 are conspicuous, and the mesenterial filaments on protomesenteries III and on metamesenteries 2 are long and much convoluted. At this stage the first couple of metamesenteries is already longer than the third couple of protomesenteries. The actinopharynx has increased in length, and the hyposulcus is relatively long. Hemisulci are present. The body-wall is as yet thin and its ectodermal muscular and nervous layers feebly developed. There is no terminal pore. Germ-cells, not much advanced beyond those of the previous stage, are recognisable in the first and third metamesenterial couples. This is the earliest stage in which I could detect Carlgren's muscles, i. e. the longitudinal muscles on the posterior faces of the mesenteries in the actinopharyngeal region. A few specimens of this stage occur in the deep water catches; it is very numerous in the epiplanktonic catch.

Stage F (Pl. 3, fig. 16).—Nine couples of mesenteries. Marginal tentacles thirteen, consisting of one large directive, five couples of large and one ventral couple of small tentacles, the right being much larger than the left. Labial tentacles ten, the last couple very small, and there are traces of a sixth couple.

This stage represents a considerable advance on the preceding. The body is elongated and cylindrical, its walls are thick, and the muscular and nervous layers of the ectoderm are conspicuous. The external ectoderm of the body-wall is now full of the large nematocysts with an irregularly convoluted thread characteristic of Cerianthids. The histological differentiation of the sulcus, hyposulcus, mesenterial filaments and endoderm lining the body cavity is noteworthy. Most conspicuous is the increase in the number and differentiation of the germ-cells. In the first and third couples of metamesenteries there are now numerous ova with a large vesicular nucleus and deeply staining nucleolus. Alongside of these are groups of germ-cells in the nuclei of which mitotic figures are discernible, but the state of preservation did not admit of the study of details of the mitoses. From a comparison with later stages it is probable that these clusters of dividing germ-cells are destined to give rise to spermatozoa. The terminal pore is now present, and its time of appearance seems to coincide with the full development of the ectodermic musculature of the body-wall. The pore and the strongly developed muscles are doubtless correlated, the pore serving to relieve the internal pressure set up when the walls of the body are powerfully contracted.

Stage F is abundant in the epiplanktonic catch and also occurs in the mesoplanktonic deep-water catches.

Stages G and H.—I class under these all forms with nineteen or twenty (stage G) and twenty-one (stage H) mesenteries. In other respects there is very little difference between these two last stages. They have generally from sixteen (stage G) to eighteen marginal tentacles and ten labial tentacles. They all show fully developed Cerianthid characters, with a thick body-wall having strongly marked muscular and nervous layers—a well-marked terminal pore, and in all two couples of mesenteries, viz. the first and third couples of metamesenteries, bear gonads in a more or less advanced stage of development. These stages occur in both the epiplanktonic and meso-planktonic catches, but are relatively scarce in the former and predominant in the latter. Pl. 3, fig. 17 shows the largest and most complete example taken in the epiplanktonic catch. It is obviously much contracted in spirit and its total length is only 17 mm., the body measuring 9 mm. in length with a diameter of 2 mm. This particular example has all its marginal tentacles intact, and for this reason was chosen for illustration, but it is probably only an early condition of stage G. In the corresponding and later stages in the epiplanktonic examples the marginal tentacles are in the process of being cast off, and more or fewer are always missing. The contents of the jar consisted largely of cast-off tentacles. The mesoplanktonic examples have without exception cast off their marginal tentacles, only attenuated threads remaining here and there in a few cases. After examining with some care the cast-off tentacles and the scars marking their former attachment to the edge of the oral disc, I have come to the conclusion that the marginal tentacles in this species are autotomous, and are normally cast off when the animal approaches sexual maturity and descends from the epiplankton down to the depths. One reason for this opinion is that the edges of the scars, which are really perforations leading freely into the actinocœles, are not ragged as they would be if the tentacles had been torn off in the net, or as a consequence of the rapid change of pressure in the course of the ascent from deep water to the surface. On the contrary, there is a thickening of the mesoglœa all round each perforation, and the ectoderm passes as conformably into the endoderm at the edges as it does, for instance, in a mesenterial filament. Secondly, the cast-off tentacles show no signs of rough usage, but are mostly plump and retain their shapes, but in every case there is a marked constriction, deep enough to close the tentacular cavity, at the basal end, indicating a violent local muscular contraction which has severed the tentacle from its attachment. Thirdly, there is the consideration that these large marginal tentacles are clearly the organs of locomotion of the epiplanktonic phase of existence; from their inflated character it may be inferred that they function largely as hydrostatic organs, and they would not only be useless, but actually a hindrance to the descent of these organisms into deep water at the onset of sexual maturity.

For there is no longer any doubt that the deep-water forms are very nearly, though I cannot say quite, sexually mature, and I can corroborate and amplify Vanhöffen's (**21**) observations and remarks on this subject. I may say in this connection that it seems to me very probable that the floating actinian captured and figured, but subsequently lost, by Vogt, was really an Arachnactis albida in stage G or H. It is true that Vogt's drawing, made on board ship under conditions unfavourable for study, is obviously inaccurate. The smaller tentacles, which may be taken to represent the labials, are attached to nowhere in particular, and there are other obvious errors. But Vogt's figure bears so close a resemblance in outline to Vanhöffen's careful drawing, and the locality and conditions under which Vogt made his capture agree so well with all that we know of the distribution and habits of Arachnactis, that I cannot agree with the summary manner in which Boveri (**5**) dismissed this form as clearly belonging to some other genus, albeit subsequent authors, with the exception of Vanhöffen, have nearly universally accepted Boveri's criticism. On the other hand, it seems to me extremely improbable that Vogt really saw ciliated larvæ within the body-cavities of his specimens. What he probably saw was a bolus of food kept in motion by the cilia of the filaments. In several of my specimens there is a globular mass of half-digested food which, at a first glance, might well be mistaken for an embryo in the living form.

Vanhöffen described and figured, but none too exactly, ova in the fertile mesenteries of his specimens. As he only describes and figures fifteen marginal tentacles his examples cannot have been much older than my stage F, in which, indeed, developing ova are distinguishable, but not far advanced. Fowler states briefly that generative cells make their first appearance in his stage I, with nineteen mesenteries and some fourteen marginal tentacles. I have already shown that they are recognisable at a much earlier period. What is of more importance is that in my stage H, with twenty-one mesenteries, I have found both ova and spermaries, as shown in Pl. 4, fig. 21. The drawing (which does not include ova of the largest size) shows that the ova at this stage are in a fairly advanced stage of development with large pale vesicular nuclei, a sparse and feebly staining chromatic reticulum and a large deeply stained nucleolus. The spermaries are evidently immature, and the cells in them may be regarded as spermatogonia. In no case have I found ripe spermatozoa. These examples, all of which came from the deep-water catches, are therefore not fully sexually mature, though more advanced than any specimens of A. albida yet recorded. One would say that they cannot be far removed from sexual maturity, and the question arises whether these deep-water stages which have cast off their marginal tentacles and have twenty to twenty-one mesenteries may be regarded as the adults of Arachnactis albida? It is no longer possible to dismiss this question summarily, on the ground that these forms are pelagic and that all sexually mature and adult Cerianthids are sedentary, for we now know that this generalisation is incorrect. Gravier (**15**) has described in detail, under the name of Dactylactis benedeni, a pelagic Cerianthid taken by M. Léon Diguet in the Gulf of California. He is explicit: the spermaries in his examples contained ripe spermatozoa, so there can be no doubt that this species is sexually mature, and yet an epiplanktonic form, for it is expressly stated of M. Diguet's collections that they "nageaient en nombre considérable dans les couches superficielles, à la surface même ou à une faible profondeur . . .; la plupart d'entre eux possèdent des éléments reproducteurs complètement développés ou très voisins de l'état de maturité."

It would therefore be reasonable to claim that the mesoplanktonic and nearly sexually mature forms taken at depths of 450 and 550 fathoms off the coast of Ireland in 1911 represent the hitherto unknown adult form of Arachnactis albida.

But Carlgren (**9**) has pointed out close resemblances between the older forms of A. albida and a sedentary Cerianthid dredged from a depth of 140 fathoms in the Trondjhem Fiord, and named by him Arachnanthus sarsi. Without committing himself to a definite assertion, Carlgren considers that it is highly probable that Arachnanthus sarsi is the adult form of Arachnactis albida.

He gives the following critical characters in his diagnosis of A. sarsi (loc. cit. p. 27):

(1) Stomatodæum of medium length, with very long hyposulcus that reaches past the middle of the animal and exceeds the stomatodæum in length.

(2) The directive mesenteries comparatively long, but extending further than the middle of the hyposulcus.

(3) Protomesenteries of the second cycle of the length of the hyposulcus, probably sterile, without cnido-glandular tract.

(4) Protomesenteries III about the length of the directive mesenteries, with very short ciliated tract region and a very long cnido-glandular tract region.

(5) The metamesenteries of the second cycle almost as well developed as those of the first.

(6) The oldest mesenteries of the first and second cycle long, reaching not quite down to the aboral end of the animal, fertile, with long ciliated-tract region. The first metamesentery on each side with an acontium.

(7) The metamesenteries of the third (B) and fourth (b) cycle short, about the length of protomesenteries 3, sterile, with very short ciliated-tract region and very prolonged cnido-glandular tract; b slightly shorter than B.

(8) The musculature of the mesenteries comparatively well developed.

I have examined fifteen examples of Arachnactis albida with from eighteen to twenty mesenteries and five or six couples of labial tentacles, some by transverse sections and others by dissections, such as that shown in Pl. 4, fig. 19, and find the following points of agreement and difference between this species and Arachnanthus sarsi:

(1) The hyposulcus, or, as I shall call it in future, the hyposulculus, in A. albida reaches past the middle of the animal, but is very variable in length relatively to the actinopharynx (stomatodæum). Usually it is of about the same length; it may be a trifle longer, but is as often decidedly shorter. These differences obviously depend on the state of contraction of the example under observation.

(2) In all my examples the directive mesenteries extend to, and are somewhat prolonged beyond, the aboral end of the hyposulculus. This is a difference of capital importance.

(3) In A. albida protomesenteries II are always considerably longer than the hyposulculus, are sterile, but have a well-marked cnido-glandular tract forming a compact coil at their aboral extremities. This is a constant feature, and the transition from the ciliated-tract region to the cnido-glandular tract region can clearly be seen in sections. This is a second distinction of capital importance between Arachnactis albida and Arachnanthus sarsi.

(4) In A. albida protomesenteries III are decidedly longer than the directives, reaching aborally about as far as the end of the ciliated-tract region of protomesenteries II.

(5) In this feature A. albida agrees with Arachnanthus sarsi, and also generally in 6, 7 and 8.

The differences are sufficiently great, yet there are many points of resemblance. In Arachnanthus, as in my oldest examples of Arachnactis albida, there are twenty-one mesenteries, and in both species the three smallest do not bear filaments. In both species there is no labial tentacle over the directive actinocœle, nor over the two adjacent actinocœles. In both there is a well-developed acontium at the aboral ends of the first couple of metamesenteries. Further, it is worthy of notice that the single known example of Arachnanthus sarsi had no trace of tentacles, and I have shown that, as they approach sexual maturity, the larger forms of Arachnactis albida lose their marginal tentacles, and, as it would seem, sink to considerable depths, there to become fully mature. In most of my examples the labial tentacles were present and conspicuous, but in one or two of the largest taken in deep water these had also disappeared—whether torn off or cast off I am unable to determine.

From the evidence before me I think I am justified in constructing the following life-history of Arachnactis albida: The youngest larvæ appear at about midsummer and are epiplanktonic, swimming at or near the surface by the aid of their long tentacles (Vanhöffen, **21**). As is commonly the case in epiplanktonic organisms, they appear in shoals, in this differing from A. bournei, which in my experience never appears in shoals, but forms a somewhat infrequent element in epiplanktonic catches, and is taken in small numbers at a time. At first of small size, the larval A. albida grows rapidly, and on arriving at a stage with thirteen to fourteen mesenteries and ten marginal tentacles it feeds greedily on other organisms of the epiplankton, chiefly copepods, in the late summer months. Towards the onset of autumn it has attained nearly its full growth, having fifteen to eighteen marginal and ten to twelve labial tentacles, with twenty to twenty-one mesenteries. It then throws off its marginal tentacles and sinks towards the bottom, and by the time that it has reached a considerable depth its ovaries and spermaries are in a fairly advanced stage of development. It is adolescent, if not fully adult. This brings us to the autumn months, and it remains a matter of conjecture what form it assumes and what is its habitat during the winter. That it enters upon a period of winter rest can hardly be doubted, for the young larvæ do not reappear until the following spring. We do not know whether it buries itself in the sea-bottom, as do other Cerianthids, or whether it remains as an element of the mesoplankton near the bottom—more probably the latter, as no examples have been recorded as taken by the dredge in so well-explored an area as the banks off the south-west coast of Ireland. However this may be, it seems certain that it develops its ova and spermatozoa—like most other Cerianthids, Arachnactis albida is monoecious—in the warmer waters of considerable depths during the winter months, and in the spring the fertilised ova are discharged and the developmental cycle begins again.

If I am right in claiming, as I do, that the deep-water examples with twenty to twenty-one mesenteries, ten or twelve labial tentacles, a thick ectodermic musculature and gonads in a fairly advanced stage of development, are the adult forms of Arachnactis albida, and undergo little further change beyond the ripening of the sexual products, then this species is certainly not identical with Arachnanthus sarsi, as described by Carlgren. The differences in the protomesenteries, recorded above, are sufficient to separate the two. At the same time the resemblances are many, and one cannot doubt the correctness of Carlgren's conclusion, that "A. albida is a larval form falling under the same genus as Arachnanthus, which cannot be said of Arachnactis bournei and brachiolata." It is possible that the discovery of new examples of Arachnanthus sarsi may result in the disappearance of the anatomical differences which at present separate it from A. albida. Carlgren tells us that he "did not like to dissect fully the one specimen he had for study, especially as the mesenteries were not very well preserved." As the differences noted depend on mesenterial characters, further and better opportunities for study may show that they are non-existent.

But the possibility must not be ignored that the larvæ and adult or quasi-adult forms taken off the south-west coast of Ireland belong to a species or sub-species distinct from A. albida. In the first place, they come from a well-defined area several degrees south of the equally well-defined area of distribution of the more northern forms. Secondly, there is a considerable disparity in size. The largest examples taken by Vanhöffen measured, after preservation in spirit, 15 mm. in length of body; the marginal tentacles measured 35 to

40 mm. in length. The largest examples from the Irish collections measure 9 mm. in length of body, with a tentacle length of 22 mm. Thirdly, I could not find any trace of the deep, chocolate-brown coloration of the labial tentacles and actinopharynx described by Vanhöffen for A. albida, and also characteristic of the mouth and actinopharynx of Arachnanthus sarsi. The colour may have been dissolved out in spirit, but the body-walls retain a considerable amount of brown pigmentation, and if it persists here, why not in the labial tentacles? In all my specimens these latter are brilliantly white. There are also small anatomical and histological differences, to which I shall refer further on, and all these facts taken together warrant my describing the Irish forms as a distinct variety or subspecies under the name Arachnactis albida var. hibernica, with the characters enumerated above. I am further of the opinion that the differences in size, colour (?), distribution and minute anatomical detail are accompanied by a change of habit, and that the evidence at our disposal points to the conclusion that the adult hibernica does not bury itself in the sand, but remains a member of the mesoplankton during the ripening of its sexual products and to the end of its individual existence, which I opine terminates with the discharge of its ova and spermatozoa. In support of this view, I may remind my readers that some of the largest and most mature examples in my possession were taken in a mesoplankton net at a depth of 450 fathoms, but 111 fathoms from the bottom of the sea.

If I am right in this opinion, the life-history and habit of hibernica is intermediate between that of the epiplanktonic Dactylactis benedeni described by Gravier and that of Arachnanthus sarsi, supposing the last-named to be, as it most probably is, the adult form of Arachnactis albida. In his classical memoir on Cerianthid larvæ van Beneden has recorded the presence of germ-cells in Dactylactis armata and in Ovactis brasiliensis. In the latter species they are in a very early stage and no better developed than in my stage D of Arachnactis, but in the former species they are of sufficiently large size to indicate the approach of sexual maturity. I venture to predict that it will be found that several of the pelagic Cerianthids attain the adult condition whilst still epiplanktonic, others passing from the planktonic to the more typical sedentary habit of adult Cerianthids. If this be so—and we now know of at least one case in Dactylactis benedeni—it is only consistent with analogous phenomena in not very distantly related organisms—the Hydrozoa. In this group, as Weismann showed, the increasingly early development of the sexual products has led to a parallel change of habit in several families of the Hydroids, the advantage gained by early maturity more than compensating the disadvantage involved in the suppression of the free-swimming Medusa stage, whereby the ova were widely distributed. In the Cerianthids, the advantage gained by early maturation of the sexual products leads to a change of habit in an inverse direction. It is the sedentary form that is in process of elimination, whilst the pelagic phase assumes increasing importance. In instituting this comparison, I do not forget that Dactylactis benedeni, with its long directive mesenteries, acontia on the third as well as on the first couple of metamesenteries, and labial tentacles in the actinocœles adjacent to the directive, is structurally different from Arachnactis, and cannot be regarded as forming part of the same evolutionary series. But this is only consistent with the changes observed in Hydroids, in which group parallel changes are observed in the life-histories of several different families.

Anatomy and Histology.

This subject has been dealt with in great detail by van Beneden (**3** and **4**), Vanhöffen (**21**), and more lately and most fully by Carlgren (**8** and **9**). Though I have a few observations to offer, I shall not attempt to traverse all the ground that has already been so thoroughly explored.

Symmetry and Orientation.—It is necessary to justify and explain the position I have taken up and the terms that I have selected for use in the preceding pages, because previous authors differ very much from one another on these matters. Only recently, McMurrich (**18** and **19**) has given lengthy and most respectable arguments in favour of using the terms "dorsal" and "ventral" in a sense exactly inverse to that in which they are used by Carlgren. I have never found myself able to accept the homology, originally propounded by Caldwell and Sedgwick, and vigorously supported by van Beneden as a consequence of his researches on the development of Ceriantharia, between the successively formed actinocœles of the Anthozoa and the paired and unpaired cœlomic cavities of Annelids and Chordata. The arguments advanced in support of this hypothesis are extremely ingenious, and have a great attraction for those who conceive it to be the province of the zoologist to construct a system of speculative phylogenies. But whilst recognising the intellectual pleasure derived from this form of mental exercise, I cannot but regard it as altogether outside the domain of reality. The arguments brought forward are not susceptible of proof or disproof; they are only capable of sustaining a greater or less amount of probability, and when pushed to an extreme, the improbability largely preponderates over the probability. The "architectonic"—to borrow a word from German authors—of the Anthozoa and such phyla as the Annelida and the Chordata is so fundamentally different; the final result of the developmental history is so radically dissimilar, that I am convinced that we are dealing with things which, as the logicians say, are not in pari materiâ, and I cannot regard speculations as to the common origin of their characteristic structures as serious contributions to science, the more so because we are learning daily, from the school of genetics, that imaginary evolutionary series, apparently founded upon a series of demonstrated facts, break down altogether when confronted by the actual sequence of events as proved by experiment. The human intellect, however powerful and imaginative, is not only liable but certain to err in the attempt to reconstruct in detail long lost series of sequences, and I am disposed to say of this form of zoological speculation what Roger Bacon said long ago of other philosophical works: "Non est nisi amissio temporis studere in illis et multiplicatio ignorantiæ et causa erroris."

For this reason I must discard van Beneden's identification of anterior and posterior, dorsal and ventral surfaces. It does not correspond to the natural position assumed by the animal, for Arachnactis does not swim, as van Beneden supposed, mouth downwards, but mouth upwards. Thus Vanhöffen says: "Die weissen runden Randtentakeln mit bräunlicher Spitze sah man weit ausgebreitet an der Oberfläche des Wassers während der rübenförmig langgestrechte oder kugelig contrahirte Körper senkrecht dazu herabhing." It is better, I think, to adopt the conventional orientation originally proposed by Kölliker, and to use the terms "dorsal" and "ventral" in the sense adopted by him. I should have preferred to adopt a more logical system and to make use of the descriptive terms "sulcar" and "sulcular" to define the opposite ends of the long axis of the actinopharynx, as I proposed many years ago, but the majority of authors seem to prefer the conventional though meaningless nomenclature, and it is best to conform to the prevailing practice.

The question then arises which is the "dorsal" and which the "ventral" aspect of a Ceriantbid? I have no hesitation in ranging myself on the side of Carlgren, McMurrich's special pleading notwithstanding. The single œsophageal groove of Cerianthids is "dorsal," and the opposite aspect, where new metamesenteries are added, is "ventral." Hence the œsophageal groove of Cerianthids corresponds to the sulculus, not to the sulcus, of Hexactinians, and I have called it by that name. Is not Carlgren himself inconsistent when he applies the name "siphonoglyph" to the dorsal groove of Cerianthids? The term "siphonoglyph" was invented by Hickson to denote the ventral groove of Alcyonarians. For the rest, Carlgren's argument on this subject appears to me irrefutable, and it is not necessary for me to repeat it. I only take this opportunity of stating that I fully confirm his account of the arrangement of the musculature on the faces of the mesenteries in Arachnactis. The longitudinal muscles are on the ventral faces of all the mesenteries, including the directives. I had made drawings illustrating this point, but they so precisely resemble Carlgren's (**9**, tab. v, fig. 8) that it is not worth while to publish them.

Having settled the use of the terms "dorsal" and "ventral" it is necessary to explain my use of the terms "right" and "left." I use them in a purely descriptive sense, the animal being supposed to be placed mouth upwards with the dorsal or sulcular aspect furthest from the observer. They thus correspond with van Beneden's use of them, but without any implication as to anterior and posterior extremities.

Lastly, I am in full agreement with Carlgren's view that the first three couples of mesenteries, counting from the dorsal side and including the directives, are the primary or protomesenteries, and that all the succeeding couples are secondary or metamesenteries. His arguments in favour of this view seem to me unassailable.

The Mesenteries.—As regards their general arrangement, succession, and differentiation into alternate fertile and infertile couples, I can only confirm the accurate observations of Boveri, Faurot, van Beneden and Carlgren: Vanhöffen's attempt to demonstrate an entirely different sequence of mesenterial development to that described by Boveri and van Beneden has already been sufficiently criticised by the two last-named authors. As I have pointed out, the full histological differentiation of the mesenteries begins at the close of stage C, and is nearly complete in the protomesenteries and in the first couple of metamesenteries in stage D. Prior to this the mesenterial filaments, excepting those of protomesenteries II, are not more differentiated than they are in the second and third couple of metamesenteries as shown in Pl. 4, fig. 20. It will be noticed in this figure that the endoderm on the lateral faces of the mesenteries is still very thin. It is at this stage that I find the first evidence of the larvæ beginning to feed on Copepods, and immediately afterwards the endoderm takes on what may be called its adult character, and consists of very long highly vacuolated cells, with various inclusions, such as nematocysts and trophocytes, which I shall deal with further on.

The section in Pl. 4, fig. 20, passes through the hyposulculus, to which the directive mesenteries are attached. The second couple of protomesenteries have the characteristic T-shaped filament of the ciliated-tract region. Of the third couple of protomesenteries the section through the right-hand member passes through the ciliated tract region, which, it will be observed, is slightly different in outline from the corresponding region in the second couple of protomesenteries. On the left hand, the section passes through the cnido-glandular tract region of the third couple of protomesenteries. The first couple of metamesenteries have conspicuous T-shaped filaments, but the second and third couples of metamesenteries have as yet small and undifferentiated filaments.

Without going into further details, I can confirm all that previous authors have observed. As successive couples of metamesenteries are added in the ventral actinocœle they are differentiated into alternate fertile and sterile couples, the couples bearing an odd number being fertile, those bearing an even number, sterile. The two kinds can easily be distinguished, at a short distance below the enterostome, by the different shape of the filaments in cross section. The filaments of the fertile mesenteries are invariably T-shaped and the angles formed by the limbs of the T are lined by relatively large cubical cells, with large deep-staining nuclei, and bearing long cilia. These are the "ciliated tracts" of the authors, but a more appropriate name would be "ciliated grooves." In all my examples with sixteen mesenteries and upwards I have found germ-cells, in a more or less advanced stage of development, in the first and third couple of metamesenteries, very early germ-cells in the fifth couple of metamesenteries, but none in the second couple of protomesenteries. I have also noticed a distinction which seems to have escaped the attention of other observers. In the second couple of protomesenteries and in all the metamesenteries designated by an odd number, except the very youngest, the lateral endoderm of the mesenteries contains large sausage-shaped nematocysts, different from those occurring in other parts of the body. The alternating couples of mesenteries, i. e. the sterile couples, are altogether devoid of these nematocysts. By way of contrast I have placed one of these nematocysts alongside two large nematocysts, one from an acontium, the other from the cnido-glandular tract of a filament, in Pl. 3, fig. 18 A, B and C. The nematocysts from the endoderm of the second couple of protomesenteries and from the fertile metamesenteries are half as long again as the others, are curved, and the axial thread and spiral filament are placed eccentrically nearest the convex side of the capsule. They stain bright green in picro-indigo-carmine, the capsule and its contents of a transparent light green, the thread and axis darker green, and are very conspicuous objects. In sections stained with hæmatoxylin and eosin they are coloured pink, but are not so clearly differentiated as by the picro-indigo-carmine stain. What their function and significance may be I cannot tell, but since they are constantly present in certain couples of mesenteries and as constantly absent in the other alternating couples, they must be correlated in some way with the other structural and physiological characters that distinguish the alternate couples. The only other general remarks that I have to make about the mesenteries are the following:

The directive or first protomesenterial couple are short, but continued a short distance below the lowest limit of the hyposulculus, and are attached to the short hemisulci. I confirm Carlgren's observation that the so-called filaments on these mesenteries are really continuations of the hemisulculi: in fact "hemisulculus" is only another term for the somewhat specialised filament of the directives. Below the hemisulculi the directives have no filament, nor even a craspedion. Their edges are covered by slightly enlarged but otherwise undifferentiated endoderm cells.

The second couple of mesenteries has a very long ciliated-tract region, extending far below the lowest limit of the hyposulculus, towards the aboral end of the body. This ciliated-tract region is quite straight in fully expanded examples, but in contracted examples may be so sinuous as to resemble the convolutions of the cnido-glandular tract-region. At their aboral extremities the ciliated-tract region of the second protomesenteries passes abruptly into a cnido-glandular tract region, which forms a conspicuous and compact coil. The protomesenteries of the second couple never contain germ-cells, but, as I have observed, always exhibit the large sausage-shaped nematocysts in the endoderm covering their faces. This couple is in many ways intermediate between the fertile and sterile couples of the metamesenterial series, and it is perhaps a legitimate speculation that in some ancient "Cerinula" ancestral form with only three couples of mesenteries they were the fertile couple, but have since lost this function.

The third couple of protomesenteries has a short ciliated-tract region and a long, much convoluted, cnido-glandular tract region. They are of constant length, reaching aborally as far as the ciliated-tract region of the second protomesenterial couple. The first couple of metamesenteries is the longest in the body, extending nearly as far as the aboral pole. These mesenteries are always fertile, have a very long ciliated-tract region, normally straight, but puckered into numerous coils in contracted examples; no cnido-glandular tract region, and they end in the short digitiform offsets generally known as acontia.

The second couple of metamesenteries have the same structure as the third couple of protomesenteries, and are about as long as the latter. In some sectional series they appear to be rather longer.

The third couple of metamesenteries is, if anything, a trifle longer than the second couple. It is always fertile, and, saving that it has no acontia, is in every respect similar to the first couple.

The fourth couple of metamesenteries is scarcely more than half as long as the second couple, is sterile, and presents the same characters as the last-named. The sixth couple is similar, but extends a very little way below the enterostome and has a very short cnido-glandular tract.

The fifth couple of metamesenteries is shorter than the fourth, and does not contain developed germ-cells in any of my preparations. But it is possible to detect clumps of cells either closely adherent to, or included within, the mesoglœal lamina, and as these exhibit mitotic figures, they are doubtless early germ-cells. This couple of mesenteries probably becomes fertile in individuals older than any that I had for examination.

In examples in which the seventh and a member of the eighth couple of metamesenteries were present, these did not extend down to the level of the enterostome and showed no trace of filaments.

The Mesenterial Filaments.—These structures, as seen in A. albida, were concisely and accurately described by Fowler (**13**) in 1897. More recently Carlgren (**19**) has studied them in great detail, giving a comparative account of their histology in different genera and species of Cerianthidæ, and dealing with the nomenclature of the various regions into which a filament, whether of a fertile or sterile mesentery, can be divided according to its histological characters. I have not found this account easy to follow. The facts are correctly described, but the terms "region," "tract," "streak" and "groove" are so loosely defined and so capriciously applied that the meaning is often obscure. I venture to apply a revised and exact nomenclature to a summary of Carlgren's exposition. The name "craspedon" was originally given by Gosse to the whole mesenterial filament of an Actinian. It is derived from the Greek κράσπεδον, an edge or hem, and on etymological and other grounds it is better to use it in its original signification rather than limit it to any specialised part of a filament. At a later date the brothers Hertwig (**17**) demonstrated that a craspedon has not a uniform structure throughout, but differs in histological and other characters in the oral, the middle and the aboral regions of its course. In the Cerianthidæ the more aboral region is straight, or nearly so. This may be called the orthocraspedon. There follows a much coiled region, to which the name "plectocraspedon" (πλεκτός, twisted) may be given. The terminal or most aboral region, if it has special characters, may be called the "telocraspedon."

The Hertwigs further described three longitudinal streaks, stripes or tracts in the craspedon. A middle "Drüsenstreif," afterwards known as the cnido-glandular tract, and on either side of it a "Flimmerstreif," afterwards known as the ciliated tracts. It will save confusion if the term "tract" is strictly limited to these linear differentiations of the epithelium of the craspedon, and if we limit the term "region" to the three above-named divisions—the ortho-, plecto- and telocraspedon.

Carlgren shows that in Cerianthidæ the most oral region in all the mesenteries, excepting the directive mesenteries which have no true filament, is similar in structure, though, as described above, it varies considerably in length in the various couples. This orthocraspedon is more differentiated histologically than the Hertwigs or even Fowler described, and may be subdivided, as is shown in Pl. 5, fig. 24, into a middle portion, composed almost exclusively of granular columnar epithelial cells, each bearing a single flagellum and two lateral portions, in which spirocysts and glandular cells predominate. Carlgren calls the whole of the middle tract the "median streak," and subdivides it into a middle and two lateral portions, but proposes, as an alternative, the name "spirocyst-glandular tract." Alternative names are objectionable, and it will be well to adopt and use only the term "spirocyst-glandular tract." The orthocraspedon in the Cerianthidæ is straight or at the most slightly sinuous; its median tract is a spirocyst-glandular tract, and the two lateral ciliated tracts always co-exist with the last-named. It forms the oral region of all mesenteries except the directives, is very long in protomesenteries II and in metamesenteries 1, 3, 5, but is short in protomesenteries III and in metamesenteries 2, 4, etc.

Macroscopically the region of the plectocraspedon is conspicuous by the convolutions into which it is thrown; microscopically it is characterised (1) by the absence of the lateral ciliated tracts; (2) by the character of the middle tract, which is more or less tongue-shaped in section, and its epithelium contains an abundance of large, coarsely-granular gland-cells, and the spirocysts are replaced by large, thick-walled nematocysts of the type shown in Pl. 3, fig. 18B, almost to the exclusion of the former. For this Carlgren reserves the name "cnido-glandular tract," and though there is a certain inconvenience in thus limiting the significance of a term originally used in a more general sense, it will be best to follow him. In sections stained with picro-indigo-carmine or with hæmatoxylin and eosin, the cnido-glandular tract stands in sharp contrast to the spirocyst-glandular tract. In the former the gland-cells are highly eosinophilous or stained bright green with picro-indigo-carmine, whereas the gland-cells of the lateral areas of the spirocyst-glandular tract are but feebly eosinophilous and give a light indigo-blue colour with picro-indigo-carmine.

The telocraspedon is the aboral region in which only a spirocyst-glandular tract remains, the lateral ciliated tracts having disappeared. It corresponds to the "craspedion" region of Carlgren. In Arachnactis albida, as a rule, mesenteries that have plectocraspeda, viz. protomesenteries II and III and metamesenteries 2, 4, etc., have no telocraspedal region, and vice versâ, those that have telocraspeda, viz. metamesenteries 1 and 3, have no plectocraspeda.

But in one of my series the left member of the first metamesenterial couple has a very short convoluted region some way above the acontium, and this has in part the characters of a plectocraspedon in that it exhibits the large, thick-walled nematocysts and eosinophilous gland-cells characteristic of the latter region. This exceptional differentiation, however, is absent from the other member of the couple. Carlgren tells us that the telocraspedal region co-exists with the plectocraspedal in certain mesenteries in Cerianthus lloydii, vogti and americanus, and in all the mesenteries but the directives in Botrucnidifer norvegicus.

Carlgren (**9**, text-figs. 8, 9, 10, p. 61) describes and figures transverse sections through the orthocraspeda of Arachnactis albida, Cerianthus lloydii and Botrucnidifer norvegicus, and says that there is a regular gradation in the form of the mesoglœal lamella where it broadens out in the craspedon in these and other Cerianthids. I cannot speak for species which I have had no opportunity of examining, but would point out that at least two of his grades are represented in Arachnactis albida. Pl. 5, fig. 24, is a careful drawing of a section through an orthocraspedon of a fertile mesentery (metamesentery 1) in this species. It has all the characters shown in Carlgren's text-fig. 9, and stated by him to be characteristic of the species. But Pl. 5, fig. 25, is a drawing of a section through a sterile mesentery of the same individual as that from which Pl. 5, fig. 24, is taken, and it has much more nearly the characters of Carlgren's text-fig. 8, which he describes as characteristic of Cerianthus lloydii. A further reference to Pl. 4, fig. 20, shows that in newly-developed mesenteries the shape of the spirocyst-glandular tract and the club-like termination of the mesoglœal lamina nearly resembles the condition said by Carlgren to be characteristic of Botrucnidifer norvegicus, so all three grades, and, indeed, many intermediate ones, are represented in one species—A. albida. Generally, in A. albida, the orthocraspeda of the sterile mesenteries have the appearance in transverse section shown in Pl. 5, fig. 25, those of protomesenteries II and the fertile metamesenteries are broader, and the spirocysts and gland-cells are more distinctly grouped at the edges of the spirocyst-glandular tract, as shown in Pl. 5, fig. 24. But the actual shape varies very much according to the state of contraction. Very commonly the edges of the spirocyst-glandular tract are turned up so as to enclose a deep groove lined by the ciliated cells of the central portion, and in another part of the same craspedon the edges are depressed as shown in Pl. 5, fig. 24. The shape and relative thickness of the mesoglœal lamina also varies with the state of contraction, and if, as I think is the case, the mesoglœa itself is contractile, or at any rate very plastic and liable to great changes of shape as a result of the contraction of adjacent tissues, it is evident that no reliance can be placed on the form of the orthocraspedon as a specific criterion.

Carlgren also deals at some length with the origin of the craspedon, and subscribes to the opinion already expressed by Fowler and others, that not only the spirocyst-glandular and cnido-glandular tracts, but also the lateral ciliated tracts, are direct continuations of the actinopharyngeal epithelium, and therefore ectodermic. The ciliated tracts, he says, are direct continuations of the actinopharyngeal furrows, of which the cells lining the deeper parts of the grooves change their character in the most aboral part of the actinopharynx and take on the character of the cells of the ciliated tracts. At the point of departure of the mesentery from the lower edge of the actinopharynx, the modified ectoderm in the bottoms and a portion of the sides of the furrows of the latter passes over into the ciliated tracts. As I have argued that the analogues of the ciliated tracts and also the acontia of the micromesenteries of Decaphellia psammomitra are endodermal in origin, I have paid special attention to this point. The facts are as Carlgren describes, but the actual conditions not quite as simple as would appear from his description and figures (loc. cit., tab. iv, figs. 1, 2 and 3). The so-called enterostome of Arachnactis, and of Ceriantids in general, is not a simple opening at the bottom of a tubular actinopharynx, but the walls of the latter diverge from the mid-ventral line like the folds of a pair of curtains, and the edges of the folds are both festooned and everted in such wise that their edges are turned up and project into the actinocœles. This is illustrated in Pl. 5, fig. 26, which is a tracing of a section cutting through the descending wall and the upturned lower edge of the actinopharynx. The outer face of the latter is covered by bands of modified ciliated cells, here indicated by a thick black line. The details of these cells are given in Pl. 5, fig. 29.

Pl. 5, fig. 27, is a drawing of a section somewhat lower down which passes near the edge of the fold of the upturned actino-pharyngeal wall. The bands of ciliated cells are interrupted at the points where the furrows of the actinopharynx are curving round its everted lower edge. In Pl. 5, fig. 28, the actinopharyngeal ridges are passing into the craspeda and are separate from one another. The groove-shaped ciliated tracts formed by the disconnected portions of the ciliated band are now apparent. Inter alia these sections show the shift of the attachments of the mesenteries from the grooves to the ridges of the actinopharynx as van Beneden described.

It is evident that the bands of ciliated cells mark the line where the ectoderm passes into the endoderm, and if we follow them down the craspeda as the ciliated tracts, we always find them forming the boundary line between the two germinal layers. Eventually, in the region of the plectocraspedon and telocraspedon, they die out and the boundary-line becomes indistinct. These relations may be understood by reference to the diagram Pl. 5, fig. 30, in which the lighter tone represents the endoderm, the darker tone the ectoderm; the thick black line represents the modified epithelium of the ciliated tract, and the broken lines the insertions of the mesenteries on the actinopharynx and craspeda. One half of the animal is represented, the actinopharynx with its everted rim being completely flattened out in one plane. Whether the modified cells of the ciliated tracts should be referred to ectoderm or endoderm is an open question, and perhaps of no very great importance. What is clear is that they indicate the boundary-line between the two layers, and if there is an invaginate gastrula in the Cerianthids—a matter on which we have no exact information—they may be regarded as a differentiation of the lip of the blastopore pushed into the interior of the animal by the secondary invagination that forms the actinopharynx, and further drawn out into ribands by the downgrowths from the actinopharynx which attach themselves to the edges of the mesenteries and form the craspeda.

The Acontia.—I have been successful in obtaining good and truly transverse sections of these structures, and have to make the following remarks about them. They appear to me to be direct outgrowths of the telocraspedon (craspedion of Carlgren) of the first couple of metamesenteries, and I can find no trace of the ascending and descending limb round the edge of an attenuated outgrowth of the mesentery as described by Carlgren. Pl. 4, fig. 22, represents a highly magnified section through the middle of an acontium; Pl. 5, fig. 23, the origin of an acontium from the telocraspedon under less magnification. As Carlgren describes, the bulk of the acontium is made up of large goblet-shaped gland-cells, of which the contents stain greenish-yellow in picro-indigo-carmine, but intensely blue-black in hæmatoxylin, this colour-reaction indicating that they are mucus-secreting cells. Interspersed among them are a few goblet-cells with conspicuous eosinophilous granules. Nematocysts are scanty; those that are present are of the thick-walled variety and resemble those found in the cnido-glandular tracts; one of them is shown in Pl. 3, fig. 18A. The large curved sausage-shaped nematocysts so abundant in and characteristic of the endoderm of the sides of protomesenteries II and the fertile metamesenteries are never present in an acontium, but at the bases of these structures the mesentery, devoid of any craspedal thickening and with its edge simply covered with unmodified endoderm cells, is continued for some little distance aborally, and here the large sausage-shaped nematocysts are abundant. In a prolonged search with an immersion lens I have failed to find any spirocysts in the acontia, though, as shown in Pl. 4, fig. 23, they are present in the telo-craspedon right up to the point of origin of the acontium. The presence of thick-walled nematocysts and the absence of the characteristic endodermal sausage-shaped nematocysts indicate that the acontium is, as Carlgren urges, an ectodermal outgrowth, and it more nearly resembles a cnido-glandular than a spirocyst-glandular modification of the craspedon. It is clearly mucus-secreting rather than urticatory in function. But as far as my own observations go, I cannot find in it any of the characters of a craspedoneme. Nevertheless, Carlgren, who was able to compare these structures in different species, is very possibly right in his contention that they are by origin craspedonemes, i. e. narrow riband-like outgrowths of the mesentery with ascending and descending limbs of the craspedon along their edges. In, this they differ from the acontia of Sargartiidæ and Phelliidæ, but the latter are not so very different in origin, for I think it must be conceded that they also are very narrow riband-like outgrowths of the mesenteries, but with a craspedal outgrowth along one edge only. Unquestionably, the so-called acontia of Cerianthids have arisen quite independently of the true acontia of Sagartiidæ and differ from the latter both in structure and in function, but they are remarkably similar both in general appearance and in situation.

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EXPLANATION OF PLATES 3, 4, and 5,

Illustrating Prof. G. C. Bourne's paper on "Observations on Arachnactis albida, M. Sars."

Lettering.

ac. Acontium. *aph.* Actinopharynx. *cn. g.* Cnido-glandular tract. *cr.* Craspedon. *c. t.* Ciliated tract. *en.* Endoderm. *hy. sc.* Hyposulculus. *lb. t.* Labial tentacles. *m.* Muscular layer. *m. d. t.* Median marginal or directive tentacle. *mgl.* Mesoglœa. *m. t.* Marginal tentacles. *n.* Nematocysts. *o. cr.* Orthocraspedon. *ov.* Ova. *p. cr.* Plectocraspedon. *sc.* Sulculus. *sp.* Spirocysts. *sp. g.* Spirocyst-glandular tract. *sp. z.* Spermatogonia. *t. cr.* Telocraspedon. *t. p.* Terminal pore. The roman numerals indicate protomesenteries, the arabic numerals metamesenteries.

PLATE 3.

Fig. 1.—Arachnactis bournei, Fowler. Oral view of an individual with eleven marginal and six labial tentacles. × 16.

Fig. 2.—A. bournei. The same individual viewed from the dorsal surface. × 16.

Fig. 3.—A. albida Sars. Oral view of an individual of stage A, showing four large and two smaller marginal tentacles and two couples of short labial tentacles. × 22.

Fig. 4.—Ventral view of the same individual as depicted in fig. 3. × 22.

Fig. 5.—A transverse section through the oral cone of the individual depicted in figs. 3 and 4, showing the first and second couples of protomesenteries. Between the former is an epithelial thickening, the rudiment of the marginal directive tentacle. × 56.

Fig. 6.—A transverse section through the same individual taken just below the enterostome, showing three couples of protomesenteries and the two first couples of metamesenteries; the last couple is very rudimentary.

Fig. 7.—A similar transverse section passing below the level of the tentacles but including one on the right side. Protomesenteries I have disappeared; protomesenteries II are large with conspicuous craspeda; protomesenteries III and metamesenteries 1 are still present, but the second metamesenterial couple has disappeared.

Fig. 8.—A. albida. Oral view of an individual of stage B, showing four couples of marginal tentacles and the commencing outgrowth of the marginal directive tentacle: two couples of labial tentacles. × 22.

Fig. 9.—A transverse section through the middle of the actinopharynx of the individual depicted in fig. 8, showing five couples of mesenteries, and in addition a single member of the sixth couple.

Fig. 10.—A. albida. Oral view of an individual of stage C, showing four couples of long marginal tentacles and the rudiments of a fifth couple. The marginal directive tentacle in this example is minute. There are three couples of labial tentacles. × 22.

Fig. 11.—A transverse section of the individual depicted in fig. 10, showing six couples of mesenteries and the developing right-hand member of the seventh couple.

Fig. 12.—A. albida. Oral view of an individual of stage D, showing four couples of larger marginal tentacles and the commencing outgrowth of the right-hand member of the fifth couple. In this example the median directive tentacle is large. There are three couples of labial tentacles. × 22.

Fig. 13.—Oral view of another individual of stage D, in which the marginal tentacles are inflated and inflexed over the oral disc, and have been cut short to show the mouth and labial tentacles. Note the minute median directive tentacle and the rudiments of a fourth couple of labial tentacles. × 20.

Fig. 14.—Transverse section of the individual depicted in fig. 12, showing seven couples of mesenteries; the left member of the seventh couple is incomplete.

Fig. 15.—Ventral view of an individual of stage E, showing the large size and inflated condition of the marginal tentacles, of which there are four large and one small couples. The median directive tentacle is now equal in size to the larger marginals. There are four couples of labial tentacles; the fourth couple small. The column is still short and hemispherical. × 20.

Fig. 16.—View of the oral disc of an individual of stage F: the marginal tentacles, thirteen in number, viz. one directive, five large couples and one small couple, have been cut short. The gaping mouth is surrounded by four couples of conspicuous labial tentacles and there are traces of the fifth and sixth couples.

Fig. 17.—An epiplanktonic example of stage G of A. albida, showing the now elongated and cylindrical column and the terminal pore. The marginal tentacles are long and inflated. × 4.

Fig. 18 A.—A thick-walled nematocyst from an acontium. 18 B.—A

similar nematocyst from a cnido-glandular tract. 18 c.—A sausage-shaped nematocyst from the endoderm of a fertile mesentery. all from stage G of A. albida. × 1040.

PLATE 4.

Fig. 19.—A. albida. An individual of stage H which has been opened by a longitudinal incision along the mid-ventral line and spread out flat. The preparation shows eleven labial tentacles; the marginals have been cast off. Note the actinopharynx, somewhat crumpled upwards on the right side; the sulculus and the hyposulculus; eight couples of mesenteries; the two smallest couples, being very short, are hidden under the actinopharynx. The plectocraspeda in protomesenteries II and III and on metamesenteries 2 and 4 are conspicuous, also the acontia at the bases of metamesenteries 1. × 16.

Fig. 20.—A transverse section below the enterostome of an individual of stage D. showing the hyposulculus. three couples of protomesenteries and three couples of metamesenteries, with characteristic forms of craspeda.

Fig. 21.—Portion of a section showing ova and spermatogonia of an individual of stage H. × 520.

Fig. 22.—Transverse section of an acontium showing the uniform wall of glandular cells. × 520.

Fig. 23.—A section showing the origin of an acontium from a telocraspedon. × 390.

PLATE 5.

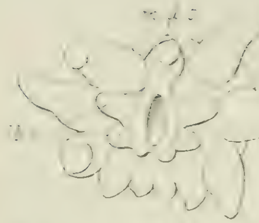
Fig. 24.—A section through the orthocraspedon of a fertile mesentery showing the broad spirocyst-glandular tract divided into a median ciliated tract and two narrower lateral tracts containing gland-cells and spirocysts. × 220.

Fig. 25.—A section through the orthocraspedon of a sterile mesentery, for comparison with fig. 24.

Figs. 26, 27 and 28.—Sections through the lower edge of the actinopharynx and the enterostome of an individual of stage C. showing the courses of the ciliated tracts. here represented by a thick black line. in this region. × 80.

Fig. 29.—A longitudinal section of a ciliated tract showing the columnar ciliated cells of which it is composed. × 520.

Fig. 30.—A diagram illustrating the relation of the ciliated tracts to the actinopharynx and craspeda. The lighter shade represents endoderm. the darker shade ectoderm: the thick black lines represent the ciliated tracts and the broken lines the insertions of the mesenteries on the actinopharynx and craspeda.



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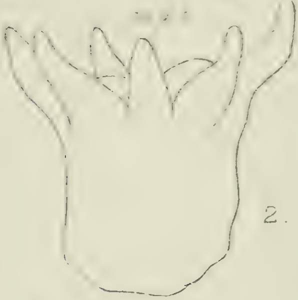

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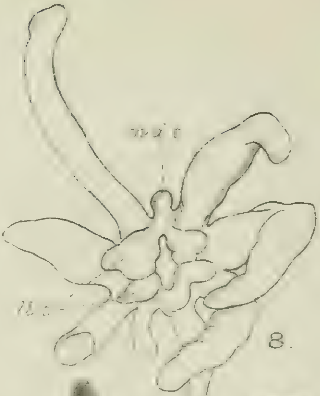

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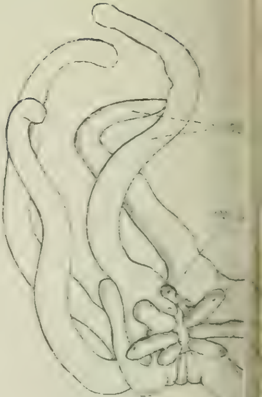

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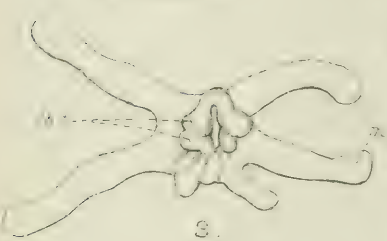

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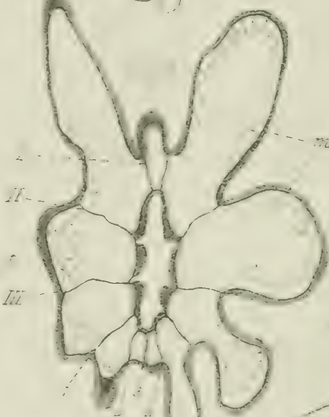

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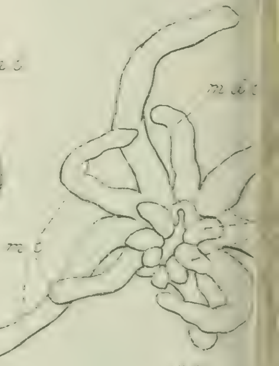

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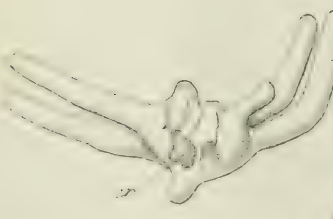

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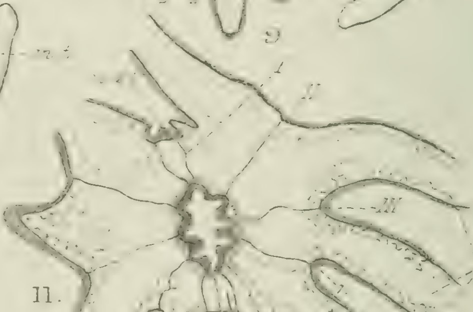

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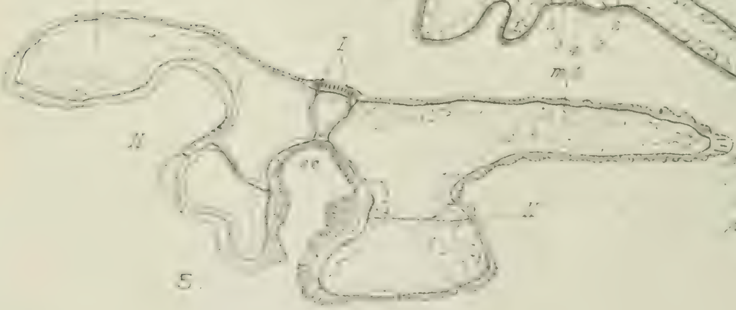

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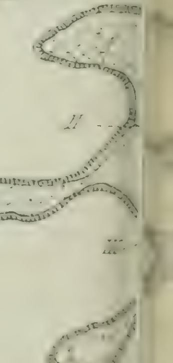

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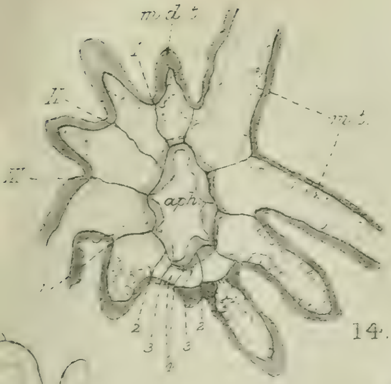

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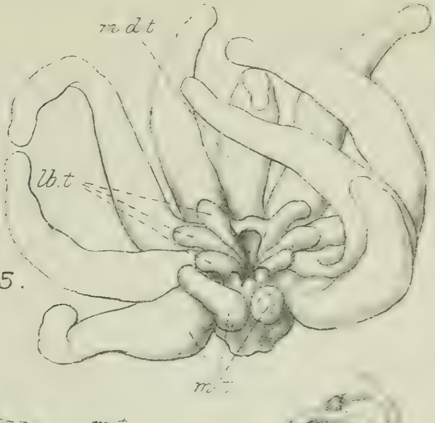

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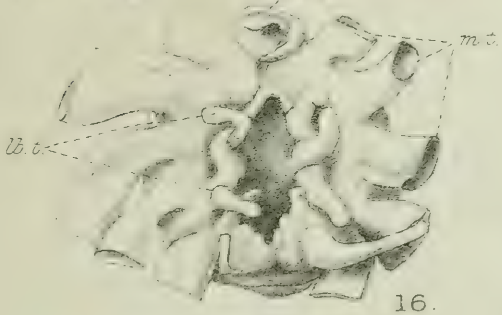

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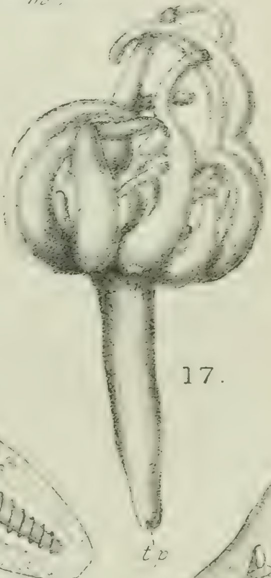

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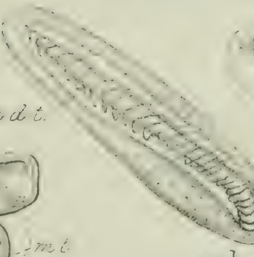

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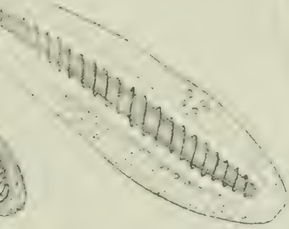

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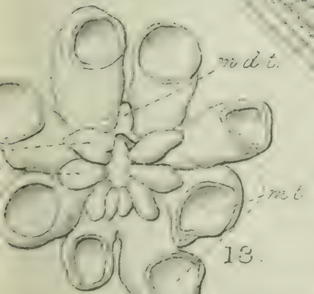

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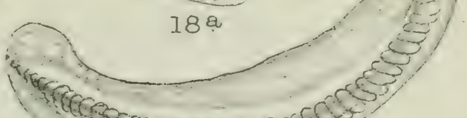

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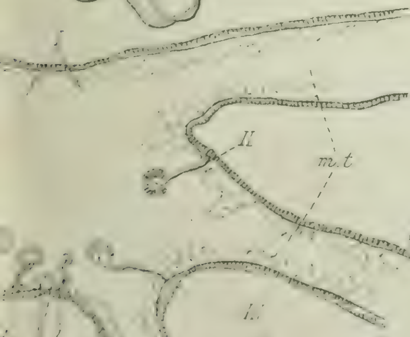

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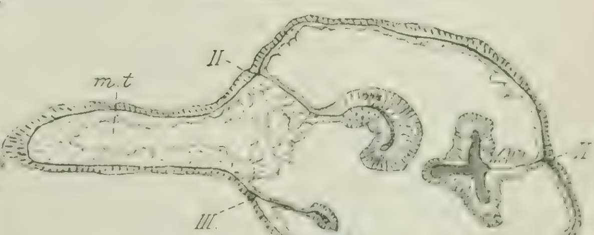

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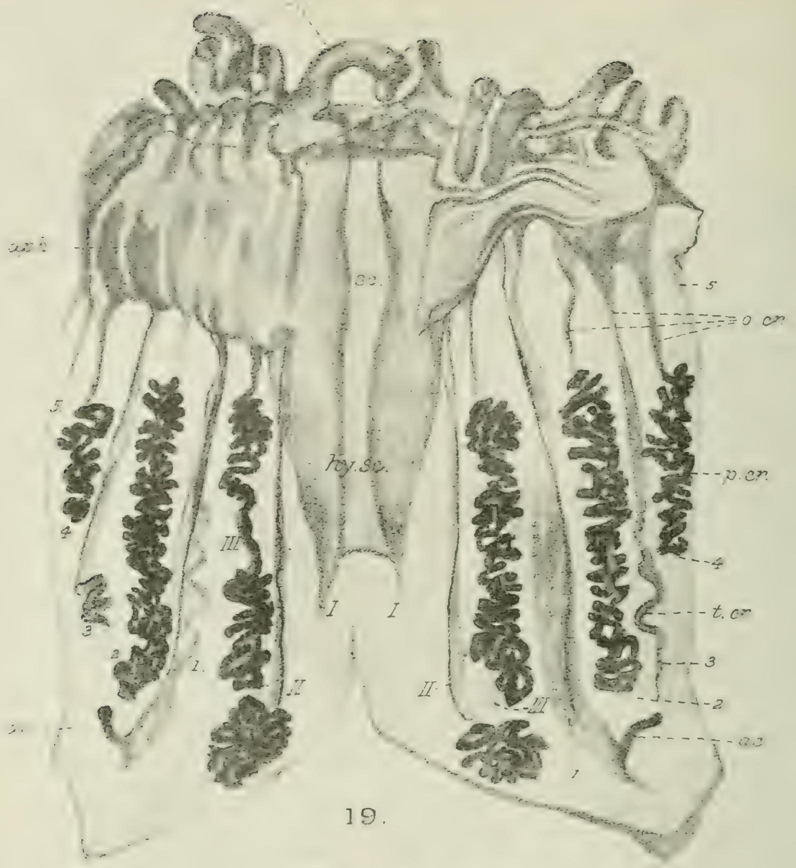

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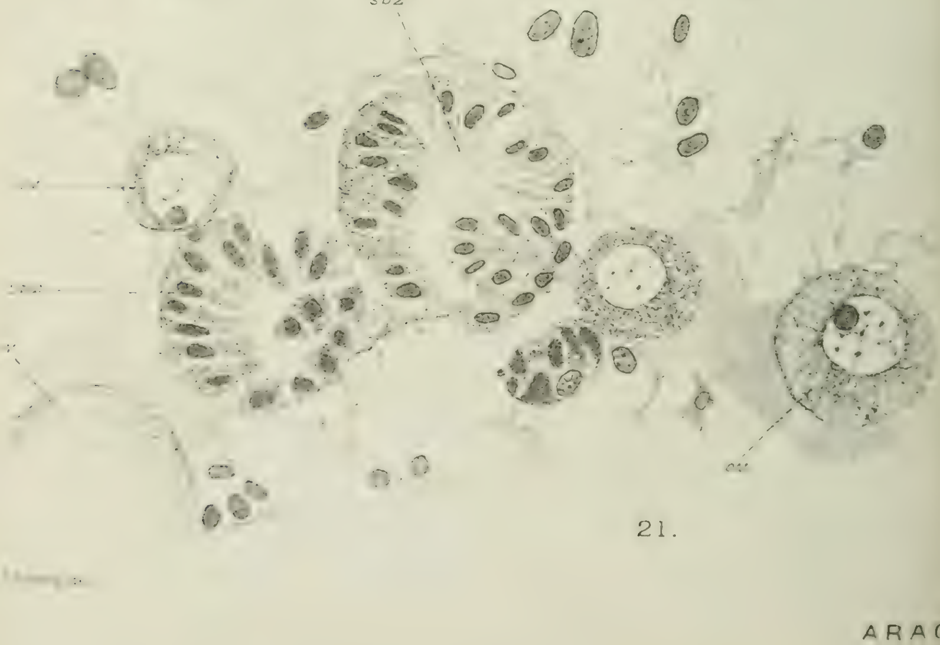

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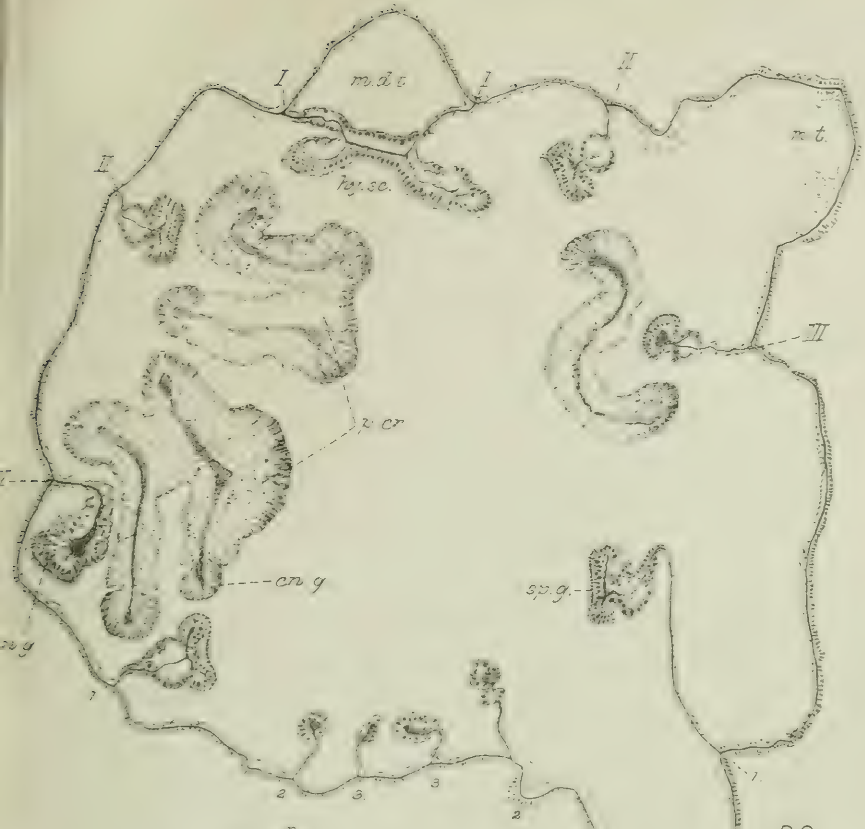

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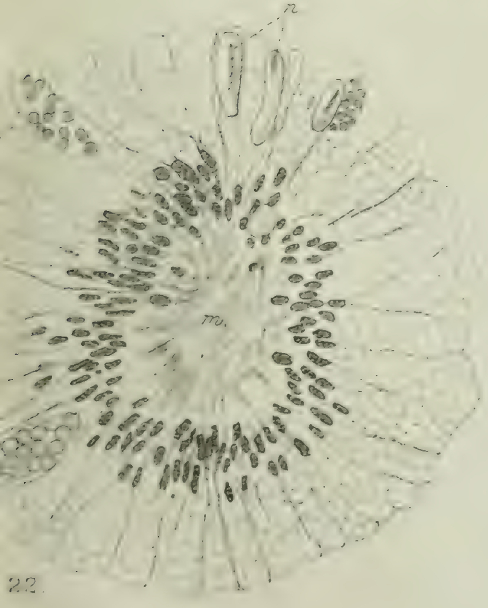

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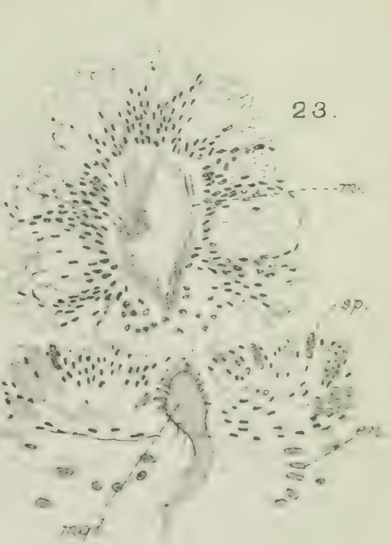

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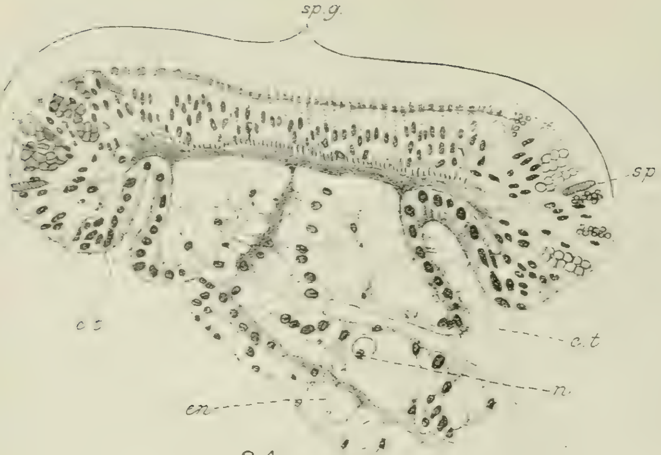

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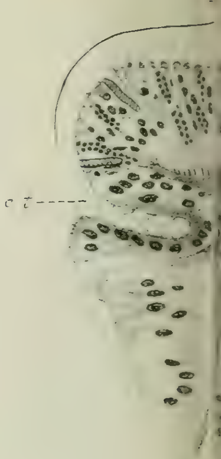

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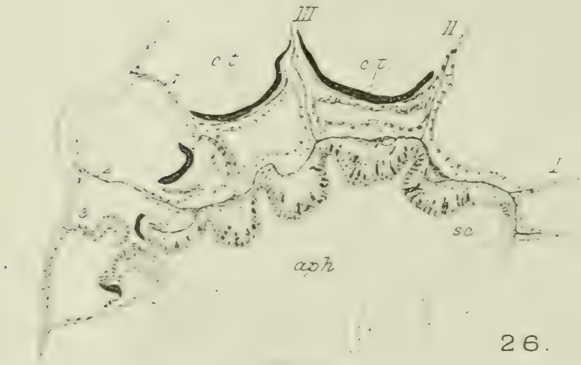

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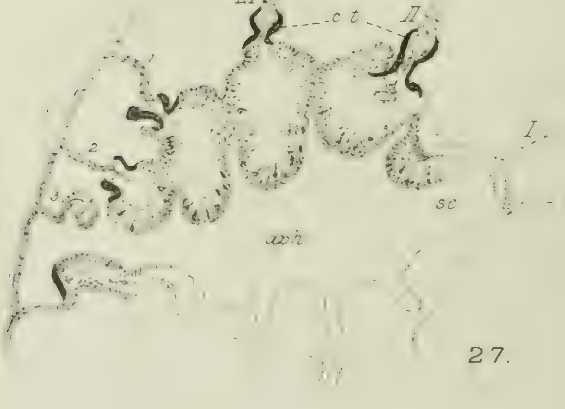

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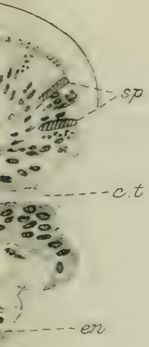

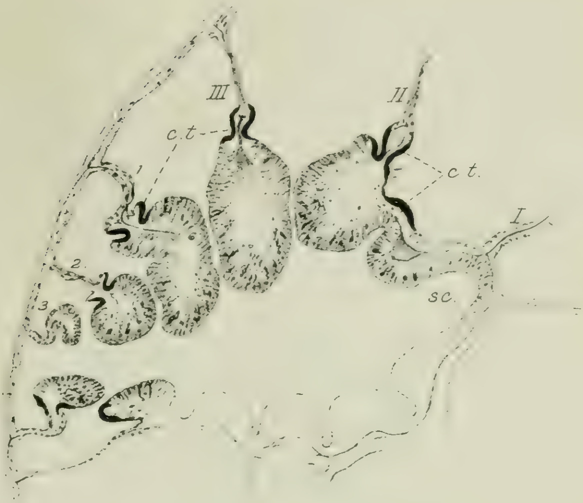

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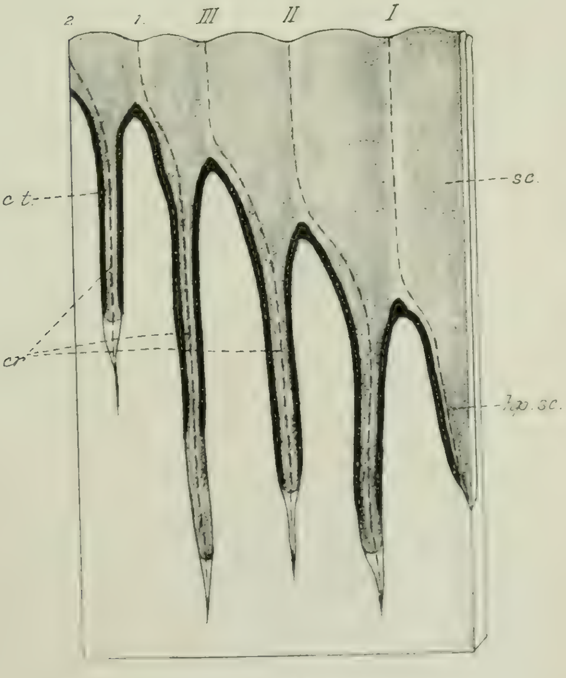

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On a New Type of Nephridia found in Indian Earthworms of the Genus Pheretima.

By

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of the Zoological Department, Muir Central College,
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With Plates 6, 7 and 8 and three Text-figures.

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1. INTRODUCTORY.

HITHERTO zoologists have recognised in Annelida in general and Oligochæta in particular two types of nephridial system.

namely—(1) the ordinary or meganephric kind, consisting of a relatively few large and separate nephridia, segmental in arrangement, which open separately to the exterior, and (2) the plectonephric or diffuse variety, in which the nephridia are numerous, of small size, and described as being connected with one another by a network of ducts possessing many openings on the surface of the skin.[1] The meganephric type is found in all Polychætes (a few with protonephridia in the adult but the majority possessing nephridia with nephrostomes opening internally into the cœlom), in all Echiuroids (with nephridial funnels opening into the cœlom internally), in most Hirudinea (always with nephridia closed at their internal ends, the funnels, where they occur, never opening into the nephridial canals), and in many Oligochætes (always with open nephrostomes). The plectonephric type, on the other hand, has been described only among Annelids in certain Oligochætes and Hirudinea (e.g. Pontobdella), and we must conclude from the descriptions available that in essential features it resembles the type of excretory system found in many Platyhelminthes (e.g. Trematodes), the sole difference being that in the latter group discrete nephridia are not present (the funnels only being represented by flame-cells), and the system of ducts rarely has more than one or two separate openings to the exterior. In both types of nephridial system, however, meganephric and plectonephric, the outer ends of the nephridia have, with but few exceptions, always been described as opening to the exterior viâ the skin.

While undertaking the accurate anatomical description of one of the commonest Indian earthworms, Pheretima posthuma (L. Vaillant), supposed to possess a typical plectonephric type of nephridial system, I ascertained that no existing description applied to the condition of things found in this species; in fact, assuming existing descriptions of the plectonephric nephridial system to be correct, the only course to adopt is to consider the nephridial system of Pheretima posthuma and other species as a distinct third type of nephridial system which is here described for the first time. Since the essential feature of this third type is that, apart from the microscopic integumentary nephridia which open through the skin, all the numerous septal and pharyngeal nephridia are connected with a system of ducts, which open, not on the surface of the skin, but into the intestine and other regions of the gut (buccal cavity and pharynx), I propose to distinguish this type of nephridial system by the term "enteronephric" suggested to me by Dr. Woodland.

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[1] Cf. Sedgwick's 'Text-book of Zoology,' vol. i, pp. 498–502.

Before proceeding to describe this "enteronephric" type of nephridial system, I will provide a brief historical summary of previous descriptions of the plectonephric type so far as these are known to me from the very limited library resources available in the United Provinces.

I may add here that I am deeply indebted to Dr. W. N. F. Woodland, Professor of Zoology at the Muir Central College, Allahabad, for the keen interest he has all along taken in my work. He has made valuable suggestions, and also found time to read through and correct the manuscript of this paper. My grateful acknowledgments are also due to my professor Lt.-Col. J. Stephenson, D.Sc., Principal, Lahore Government College, for acquainting me with the literature on the subject and sending me nicely preserved specimens of different species of earthworms, and also for his constant encouragement given to me.

Both Dr. Woodland and Col. Stephenson have examined my slides and other preparations and have confirmed all my more important statements. I have their permission to mention this fact.

2. HISTORICAL.

The earliest statement concerning a nephridial system of a kind different from the ordinary meganephric was made by Perrier, references to whose work and that of other previous writers are given by Beddard (**3**, 1895), from whose account I quote. It is noteworthy that Perrier described his new type of nephridial system in Pheretima posthuma, the species I have investigated in the present paper. Perrier states that the nephridia in this earthworm—"sont ici très rudimentaires, ce qui concorde avec l'absence d'orifice extérieur attribuable à ces organes." Beddard (**2**, 1888), however, found a large number of external excretory pores (nephridiopores) in Perichæta (Pheretima) aspergillum, and remarked that the nephridia formed numerous tufts of glandular tubules closely connected with the body-wall and the septa. Moreover, he did not regard the nephridia as rudimentary as Perrier did, but believed that they were in a very archaic condition. He emphasised two points in connection with these nephridia of Perichæta, which are, "firstly, that there is a connection between the nephridia of successive segments, and secondly, that the nephridial system of Perichæta consists of a network of tubules, the connection between the nephridial tufts of successive segments being brought about by numerous tubules which perforate the intersegmental septum." Beddard figures this network of tubules on the inside of the body-wall of Perichæta aspergillum (reproduced by Sedgwick, **13**, vol. i, p. 501). He also states in his paper that there is a very much greater number of nephridial pores in the anterior segments as compared with the posterior—a feature we shall refer to when considering the integumentary nephridia (vide infra). We may also note here in passing that it is fairly easy to see the external excretory pores in Pheretima posthuma by stripping off a portion of cuticle and examining it under the microscope, and that Perrier was certainly mistaken in recording an absence of nephridiopores in this earthworm.

Perrier's statement with regard to the position and distribution of nephridia in Perichæta robusta, which applies, according to my observations, equally well to Perichæta posthuma, is as follows: "Les organes segmentaires sous forme de tubes extrêmement délicates, sont adhérents aux cloisons, ou disséminées sur la membrane péritonéale que tapisse la cavité générale." In Pheretima posthuma also we can similarly recognise septal and integumentary nephridia according as they are attached to the intersegmental septa or are adherent to the peritoneal membrane lining the inside of the body-wall; further, the two kinds differ considerably in size though alike in structure. This distinction between the integumentary and the septal nephridia does not seem to have been recognised by the earlier observers.

Just as Beddard (**2**, 1888), described the nephridial system of P. aspergillum as consisting of a network of tubules, similarly Perrier, also in giving a résumé of the anatomy of the group, regarded the nephridial system as forming a "réseau glandulaire," which appeared to him to be an indication of an incomplete suppression of these organs. Beddard (**1**, 1885), described eight nephridial tufts in each segment of Octochætus multiporus, and also later on recorded a very large number of orifices on the skin connected with these tufts. Benham found a large number of small and separate nephridia in a species of Perichæta, and Spencer (**14**, 1888), described in Megascolides australis "a network of small tubes with many external pores." This type of excretory system formed of small tubes was called "plectonephric" (plecto, I weave) by Benham, and Beddard described it as "diffuse." The essential idea associated with these terms was that of a network, and Beddard (**3**, 1895), speaking of this diffuse type of excretory system, says: "The delicate ramifying tubules especially attached to the septa can hardly be missed, at any rate in well-preserved specimens; . . . in the Perichætidæ the network is more diffuse and not broken into separate masses." At another place, writing of the "integumental nephridial network" of the Eudrilidæ, Beddard states that these tubes do not seem to be comparable to the "cœlomic network" of Perichæta, etc. He thus differentiates between an "integumental network," which is formed in the substance of the body-wall, and which he figures for Libyodrilus in his monograph (**3**, 1895), and a "cœlomic network," which is found on the peritoneal membrane lining the inside of the body-wall, and which is figured for Perichæta (after Beddard) by Sedgwick in his text-book (**13**, 1898, p. 501). We may also mention here that the "integumental network" occurs in forms (Eudrilidæ) with paired meganephridia, while the term "cœlomic network" is a synonym for the "plectonephric" type of nephridial system.

The earlier observers, therefore, considered the nephridia in question as forming a sort of diffuse network which opened to the exterior by many excretory pores on the skin, and communicated with the body-cavity by means of funnels which were present in some tubules while absent in others. Although Benham found in a species of Perichæta a large number of small and separate nephridia, still the current idea of a "network" was essentially associated with these so-called "diffuse" nephridia.

More recently, however, Beddard seems to have abandoned the terms "diffuse" and "plectonephric," since writing as recently as 1910 (**4**), he describes these nephridia as a "mass of tubules which cover the inside of the body-wall and open to the exterior by innumerable openings," and he expresses a doubt concerning their supposed reticular connections in the following words: "It is at present uncertain whether these minute tubes are connected among themselves, thus forming a network passing through the septum, or whether each tube is isolated among its fellows, and forms a distinct nephridium, of which there are many in each segment, and entirely separate." He does not even mention here the terms "diffuse" or "plectonephric." Later still, in 1912 (**5**), Beddard uses the terms "micronephridia" and "meganephridia" when describing the two kinds of nephridia in earthworms, and not "meganephric" and "plectonephric," and it would appear from his account that he regards the nephridia as discrete structures quite distinct from one another.

From a developmental point of view these nephridia were not universally believed to be "plectonephric." The origin of the micronephridia was first elucidated by Vejdovsky (vide Thomson, **17**, 1892) in Megascolides australis; according to him there is at first one simple "anlage" on each side in each segment which breaks up into separate loops, each loop becoming a separate micronephridium, without funnel (the original single funnel degenerates), and opening to the exterior. Bourne (7, 1894), found a similar development for Mahbenus imperatrix, and both Bourne and Vejdovsky are at one in denying any secondary communication between the separate nephridia. Meisenheimer (8), in 1910, denied the existence of a network altogether. He says: "Auffassung eines plectonephrischen oder diffusen Typus von Nephridien . . . wie er aber wohl hier bei den Regenwurmern in Wirklichkeit nicht besteht."

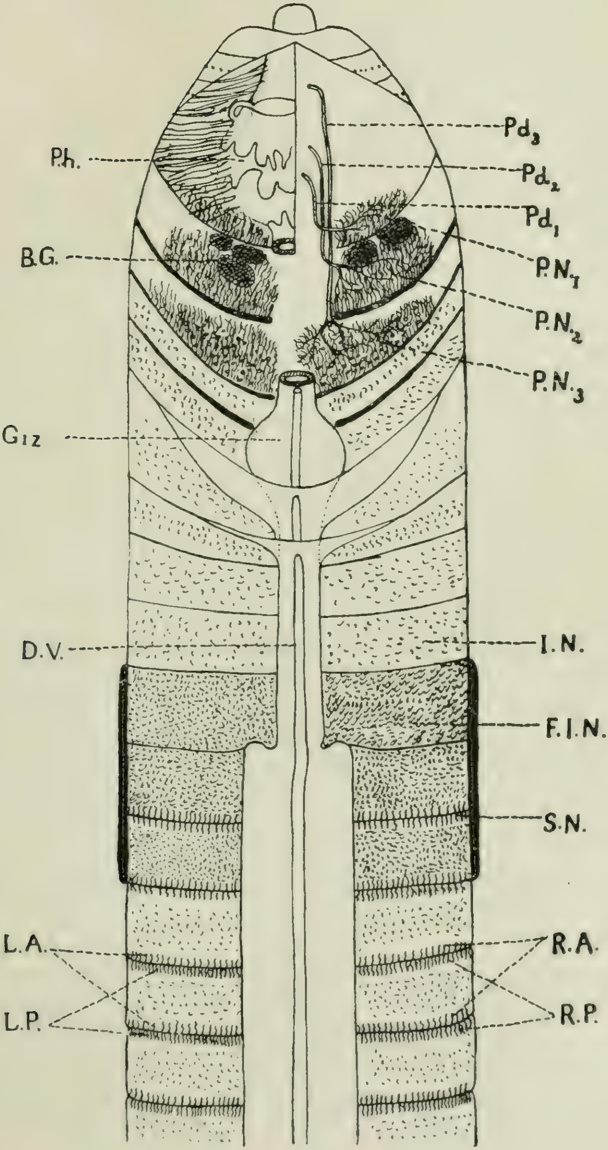
For comparison with the foregoing descriptions of the nephridia of Perichæta and allied types, I will here briefly summarise the results of my own observations on Pheretima posthuma. In the first place, these observations of mine have clearly disproved the idea of a reticulate system of ducts connecting nephridia of successive segments with each other; in other words, in the species I have examined the type of nephridial system is not plectonephric, though we may certainly retain the term "micronephric." In the second place, we can distinguish equally clearly three types of nephridia according to their size, arrangement, and place of opening of their ducts. These three kinds may be called (1) the septal, (2) the pharyngeal, and (3) the integumentary. Describing the integumentary first, we may briefly note that they are very minute structures, each of them quite separate and discrete, as was recognised by Benham. It is these only that open to the exterior viâ the skin. The septal nephridia are two to three times the size of the integumentary nephridia though similar in structure, and never open on the skin. They are also separate from one another, and are connected with a system of ducts which ultimately open medio-dorsally into the hypoblastic portion of the gut in a segmental manner. Lastly, the pharyngeal nephridia occur in bushy tufts around the œsophagus in three successive segments (fourth, fifth and sixth). Each tuft has a pair of ducts of its own, and these three pairs of ducts open into the buccal cavity and pharynx in the second and fourth segments.

Although it has long been recognised that in some Oligochætes certain nephridia do open into the alimentary canal, yet they have always been observed to open either into its anterior or stomodæal portion or into its posterior or proctodæal portion, and never into its intermediate or hypoblastic region. The former kind, i. e. those opening into the front end of the alimentary canal, have been called "peptonephridia" by Benham, and are known to occur in Enchytræidæ, Acanthodrilidæ, Eudrilidæ, Cryptodrilidæ, and Geoscolecidæ. These are supposed to possess some peptic function, and hence the name. The pharyngeal nephridia of Pheretima posthuma may possibly belong to this category. The latter kind, i. e. those opening into the rectal end of the digestive canal, have been termed "anal" nephridia, and have so far only been found by Beddard in Octochætus multiporus and by Rosa (vide Spencer, **14**, 1906) in Allolobophora antipæ. But the septal nephridia of Pheretima are peculiar in the fact already stated, viz. that their ducts open medio-dorsally into the lumen of the hypodermal gut very nearly throughout its entire length in a regular segmental manner. Beddard (**3**, 1895), writing about the peptonephridia and anal nephridia, says that they never communicate with that section of the alimentary canal which is undoubtedly of hypoblastic origin. As my description in the following pages will prove, this assertion does not apply to the septal nephridia of Pheretima.

TEXT-FIG. 1.

The general plan of the nephridial system in Pheretima posthuma. *S.N.*, septal nephridia; *I.N.*, integumentary nephridia; *P.N.*$_1$, *P.N.*$_2$, *P.N.*$_3$, the pharyngeal nephridia of the fourth, fifth, and sixth segments; *F.I.N.*, "forests of integumentary nephridia; *Pd.*$_1$, *Pd.*$_2$, *Pd.*$_3$, ducts of the pharyngeal nephridial nephridia of the fourth, fifth, and sixth segments; *R.A.*, *R.P.*, right anterior and right posterior series of septal nephridia; *L.P.*, *L.A.*, left posterior and left anterior series of septal nephridia; *B.G.*, blood-glands; *Ph.*, pharynx; *Giz.*, gizzard; *D.V.*, dorsal vessel. × 4.

TEXT-FIG. 1.



3. THE NEPHRIDIAL SYSTEM of PHERETIMA POSTHUMA.

(a) The General Plan of the System.

The nephridial system of Pheretima posthuma (Text-fig. 1) consists of three sets of tubules, each with its own system of ducts. (1) The first comprises the septal nephridia (*s.n.*), i. e. those which are attached to both sides of each of the intersegmental septa; these are the most important and the largest. They are regularly arranged on each septum throughout the length of the worm behind the fifteenth segment. These nephridia do not open on the skin, but in each segment discharge their excretory matter into a pair of septal excretory canals (*s.e.c.*, Pl. 6, fig. 1) situated on each septum, which in their turn empty themselves into a pair of supra-intestinal excretory ducts (*si.e.d.*, Pl. 6, fig. 1), which, running longitudinally along the dorsal side of the intestine, open medianly, at segmental intervals, into the intestinal lumen (Pl. 8, figs. 8 and 9).

(2) The nephridia of the second set lie just external to the œsophagus in the fourth, fifth and sixth segments, and in virtue of the place of opening of their ducts are called the pharyngeal nephridia ($P.N._1$, $P.N._2$, $P.N._3$, Text-fig. 1). They occur as paired tufts lying at the sides of the œsophagus in each of the three segments named, and are closely intermingled with follicles of "blood-glands" which are also situated in these segments. The little ductules of individual nephridia in these bunches unite together at intervals, and ultimately form a pair of long, narrow muscular ducts ($Pd._1$, $Pd._2$, $Pd._3$, Text-fig. 1) in each of these three segments. These three pairs of ducts run forwards ventro-laterally to the œsophagus and pharynx, and open into the buccal cavity and pharynx in the second and fourth segments.

(3) The nephridia of the third set, which are very much smaller in size than the septal nephridia and are adherent to and scattered over the inner surface of the body-wall (cœlomic epithelium), are described as "integumentary" nephridia (*i.n.*, Text-fig. 1). They are present in all the

segments of the worm except the first two, and they co-exist with the septal nephridia behind the first fifteen segments. Each of these nephridia opens separately to the exterior on the body-wall through a nephridiopore (Pl. 7, fig. 7).

(b) The Septal Nephridia.

(1) Position and Distribution.—The septal nephridia are attached, as their name implies, to the intersegmental septa near their outer margins close to the place where the septa join the body-wall. The septa of the first fifteen segments do not bear any nephridia, the first septum having nephridia attached to it being the septum 15/16. Behind this all the septa bear nephridia, although as we approach the posterior end of the worm the number of nephridia on each septum decreases. Each septum has nephridia attached to it on both its anterior and posterior surfaces. In a freshly-killed worm the septa present to the naked eye a shaggy appearance, and one can easily see with a hand-lens white fluffy masses attached to the septa, which are the septal nephridia. If, in a well-preserved specimen, a septum be cut off as closely as possible to the body-wall, the nephridia are also detached, since, as already stated, they are adherent to the septum. They can then be examined, along with the septum, under the microscope. On being examined under these conditions the nephridia are found to be attached to each septum in four linear series, two series being attached to the right half and the other two to the left half of each septum. The two series on each half of a septum consist of one series attached to the anterior surface of the septum and the other series to the posterior surface, i.e. an anterior and a posterior series. Each septum will therefore bear four series of nephridia (Text-fig. 1). (1) Right anterior (*R.A.*), (2) right posterior (*R.P.*), (3) left anterior (*L.A.*), and (4) left posterior (*L.P.*). As we shall see later on, the anterior and posterior series of nephridia of each half of a septum, right or left, are connected with a common duct, and therefore, on morphological grounds, we group together the anterior and

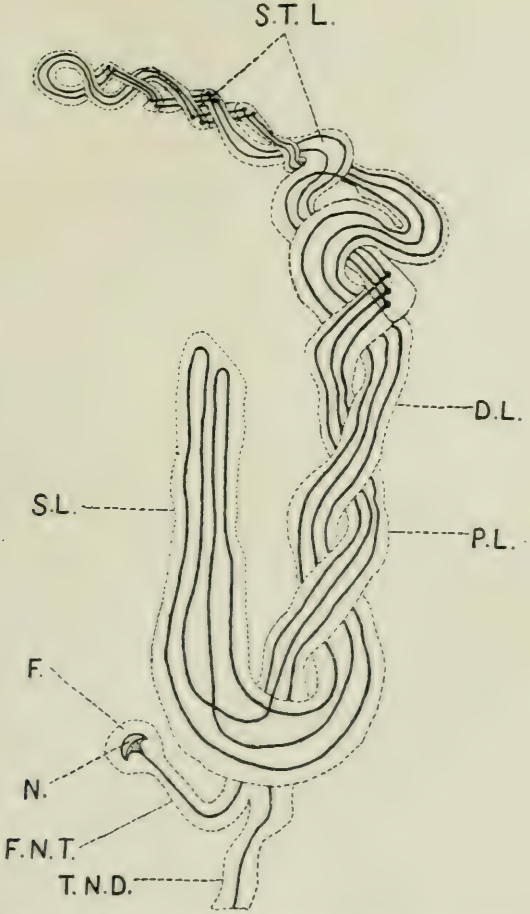
posterior series of nephridia of the right side as forming a distinct set separate from the similar series of the left side, which form another distinct set. The nephridia extend round the periphery of each septum from near the mid-ventral line of the worm almost to its mid-dorsal line.

(2) Attachment.—The line of attachment of the nephridia on each septum lies just external and parallel to the dorso-lateral vessel (the so-called "septal loop"). This blood-vessel, which plays a great part in the blood-supply of these nephridia, runs along the posterior face of each septum very near and parallel to its outer edge, i. e. the edge joining the body-wall. Each series of nephridia on each side of the septum, right or left, forms a single row with a line of attachment almost parallel to the septal loop.

(3) Number.—The number of nephridia on each septum varies within limits. On an average, there are 20 to 25 anteriorly and the same number posteriorly on each half of a septum (right or left). Each septum will thus have 40 to 50 nephridia on its anterior face and the same number on its posterior face, so that in a single cœlomic compartment we shall have 80 to 100 septal nephridia.

(4) Individuality.—Each nephridium is a minute but separate and discrete structure and there is no network of any kind connecting one nephridium with the other. Beddard (3, 1895) is thus certainly mistaken when he speaks of the excretory system of Perichæta as a "cœlomic network"; and also we cannot employ the terms "diffuse" or "plectonephric" in describing these nephridia. The idea of a reticular connection between these nephridia was probably due in the first place to the excessive minuteness of these structures, and secondly, to the fact that Pheretima and other tropical worms were unobtainable in a fresh condition by the European observers who described them. In the case of minute structures like these nephridia reliable observations cannot be made unless proper methods of fixation have been employed, and this was probably not the case with the worms examined by Beddard and others.

TEXT-FIG. 2.



A septal nephridium showing the course of the nephridial tube in it (diagrammatic). The dotted line represents the outline of the nephridium and the thick black line the nephridial tube. *F.*, funnel; *F.N.T.*, the free first part of the nephridial tube; *N.*, nephrostome; *S.L.*, the short, straight lobe; *P.L.*, the proximal limb, and *D.L.*, the distal limb of the twisted loop; *T.N.D.*, the terminal nephridial duct going to the septal canal; *S.T.L.*, the spirally twisted loop. × 276.

(5) Outline Structure and Topography of a Nephridium.—Each nephridium (Text-fig. 2) consists of (i) a funnel (*F.*) followed by a short narrow tube (*F.N.T.*), which is bent on itself before joining the body of the nephridium, (ii) the main body of the nephridium consisting of a short straight lobe (*S.L.*) and a long spirally twisted loop (*S.T.L.*). The straight lobe is rounded at its free end, and is continued at its other into one of the limbs of the long twisted loop, which is more than twice the length of the straight lobe, and (iii) a terminal nephridial duct (*T.N.D.*), which is a continuation of the other limb of the twisted loop and leads from the nephridium to the septal excretory canal.

Unlike the case in Lumbricus and Megascolides, the whole nephridium in Pheretima posthuma lies within the bounds of a single segment; in other words, it does not extend over two segments as in Lumbricus, and we do not distinguish here, therefore, a pre-septal portion of the nephridium from the post-septal portion. Beddard (**3**, 1895) says—"The nephridia are always much-coiled tubes, and they always occupy two segments, and two segments only. The internal aperture, the funnel, lies a segment in front of that which bears the external pore." He seems to regard the fact of a nephridium extending over two segments as an essential feature in the disposition of the nephridia of eathworms, and it is therefore a novel fact that in Pheretima a nephridium, including its funnel and all other parts, lies wholly within the boundaries of a single segment. Another feature already stated, and which we shall return to later in detail, is that the septal nephridia do not open directly to the exterior as they do in Lumbricus. They open, as already described, into the septal excretory canal, and that is why we have not got in Pheretima a series of nephridiopores arranged segmentally on the body-wall as we have in Lumbricus.

In brief, the whole nephridium, including its funnel, hangs freely in the cœlomic cavity, and is solely attached to the septum near its outer edge by means of the terminal nephridial duct, which runs along the septum obliquely to join the septal excretory canal (Pl. 6, fig. 1).

(6) The Minute Structure of a Single Nephridium. —Leaving aside the funnel, the first part of the nephridial tube and the terminal nephridial duct, the remaining part of each nephridium consists of a tube coiled into several loops closely bound together by membrane richly supplied with blood-vessels. The detailed account of the structure of the three parts of a nephridium is given below.

(i) The funnel (Pl. 7, figs. 3, 4 and 5). Each nephridium commences with a minute bulb-like swelling, the funnel, which has a transversely oval opening, the nephrostome (*N.*), on its ventral side. The funnel when looked at under the high power of the microscope appears slightly reniform in shape rather than rounded, and measures 28 μ in its shorter and 39 μ in its longer diameter, while it is about 15 μ in thickness dorso-ventrally.

The funnel is a two-lipped (bilabiate) structure with its upper lip of the shape of a hood; this lip is very large and prominent in comparison with the lower lip, which is very small and simple. The body of the upper lip is formed of a single large semicircular cell known as the central cell bounded by a number of marginal cells. The central cell occupies the centre and lower half of the upper lip, and has a large and prominent rounded nucleus in its centre. The outer margin of the central cell is beset with eight or nine marginal cells each with a rounded nucleus in it. All the marginal cells are equal in size, and are set in an incomplete circle round the terminal aperture of the funnel, i.e. the nephrostome, forming the margin of the upper lip. The marginal cells are profusely ciliated over the whole of their centrally directed face; the cilia are pretty long and closely set, and in whole preparations are seen to surround the top of the funnel like a halo, while in sections (Pl. 7, fig. 4) they present a characteristic bushy appearance.

The lower lip, which is not so thick as the upper, has a slightly convex border, and is made up of four or five cells, each with a deeply-staining, small elliptical nucleus. These "cells" form a compact structure, and no boundaries can be seen separating them from one another, their number being inferred from the number of nuclei. The lower lip also, like the upper, bears cilia. The actual terminal opening between the two lips, viz. the nephrostome, is, as already stated, transversely oval, and measures about 15 μ from side to side and about 9 μ dorso-ventrally. A number of cœlomic epithelial cells invest the funnel here and there.

In order to ascertain the distribution of cilia in the funnel, the latter should be examined in the fresh state when the cilia are active. If nephridia from a freshly-killed worm are examined in salt solution (·75 per cent.), the cilia are most evident in funnels which are either seen in profile or have their ventral sides uppermost. The cilia are arranged along eight or nine lines which radiate from the nephrostome or mouth of the funnel to the periphery, each line of cilia corresponding to one marginal cell. All the cilia of each line beat simultaneously towards the mouth of the funnel but alternate in movement with the cilia of the neighbouring lines. All the cilia are of the same length, and of course serve to drive the excretory matter of the cœlom into the nephrostome.

The funnel of the nephridium of Pheretima posthuma is much simpler in structure than that of Lumbricus. It is much smaller in size in accordance with the very small size of the nephridium as a whole. The central cell resembles that of the funnel of Lumbricus, but the number of marginal cells is very small, while there are no gutter-cells, so far as I can make out. Curiously enough I have never seen the "débris" of cœlomic corpuscles on the funnel of Pheretima, which fact is remarkable, since this débris is described by Benham as being a constant feature in the funnel of Lumbricus.

The funnel of P. posthuma also differs from that of Perichæta malamaniensis, described by Benham (**6**, 1891); in the latter there is no central cell, and the ciliated marginal cells form a complete circle round the terminal aperture of the funnel.

(ii) The Main Body of the Nephridium. As already stated, the body of a nephridium comprises a short, straight lobe and a long spirally twisted loop (Text-fig. 2). The straight lobe is, on an average, about 225 μ in length and 45 μ in thickness, while the twisted loop is 480 μ in length, i.e. more than twice the length of the straight lobe. The straight lobe is free and rounded at its one end, while at its other it is continued into one of the limbs (distal) of the twisted loop. The latter consists of two limbs, a proximal and a distal, spirally twisted about each other, the number of spiral twists in the loop varying from nine to thirteen (Text-fig. 2).

The funnel, together with the first part of the nephridial tube which immediately follows the funnel, is generally situated towards the side of the straight lobe away from the twisted loop. This commencing part of the nephridial tube is free for a short distance, but soon bends, to become incorporated into that limb of the twisted loop which I have called the proximal (*P.L.*, Text-fig. 2), to distinguish it from the other or distal limb (*D.L*), which is continuous with the short, straight lobe.

The nephridial tube, having entered the proximal limb of the twisted loop at its base, continues in that limb right up to the top of the loop, where it curves round, and then follows its course into the distal limb. On reaching the base of the distal limb the tube runs into the straight lobe, in which it goes to the top of the lobe, loops backwards, and then, passing through the base of the lobe, enters into the distal limb again. Here it runs parallel to its first course though in the reverse direction, and on reaching the top of the loop it bends round and runs backwards in the proximal limb until it has reached its base just about the point (*X*, Pl. 7, fig. 2), where the nephridial tube first enters the body of the nephridium. From this place (*X*, Pl. 7, fig. 2), in the base of the proximal limb the tube crosses over to the base of the straight lobe and there makes another loop similar and parallel to the first, after which the tube runs into the distal limb once again, i. e. a third time. This time it does not go right up to the top of the limb, but having gone only half the length of the limb, loops back to enter the proximal limb also a third time, and then runs towards the base of the nephridium, and is continued outside the body of the nephridium as the terminal nephridial duct.

The exact course of the tube in the nephridium can easily be followed by reference to the accompanying diagrams (Text-fig. 2 and Pl. 7, fig. 2). We may note, however, that there are, in all, three parallel tubes in each of the two limbs of the twisted loop in their lower halves, while there are only two in each in their upper halves: consequently the top halves of the two limbs are thinner than the basal halves, and the straight lobe has two loops of tubes, i. e. four tubes running parallel in it. Of these two loops one is the outer or the first loop and the other the inner or the second; the inner (*i.l.*) lies towards the side of the lobe nearer the twisted loop, while the outer (*o.l.*) loop of the tube lies away from the twisted loop. We may also observe that the terminal part of the nephridial tube leaves the body of the nephridium just about the same place where the first part of the tube enters it. Moreover, the lumen of the tube is intracellular all along, the tube being composed throughout of hollow cells joined end to end like so many drain-pipes.

(iii) The Terminal Nephridial Duct (Text-fig. 2, *T.N.D.*). The terminal nephridial duct is a continuation of the proximal limb of the twisted loop running towards the septum. Soon after leaving the nephridium it becomes attached to the septum near its outer margin just external to the dorso-lateral vessel. From this place of attachment on the septum the terminal nephridial duct runs inwards and upwards along the septum, following an oblique course (Pl. 6, fig. 1). During its course along the septum it crosses over the wall of the dorso-lateral vessel and then enters the septal excretory canal (vide infra). As the dorso-lateral vessel and the septal excretory canal lie on the posterior face of each septum, the nephridial ducts of the posterior nephridia join the septal canal without having to pierce the septum, while the ducts of the anterior nephridia generally pierce the septum and come over to the posterior surface before crossing the dorso-lateral vessel. The crossing of the dorso-lateral vessel (*dl.v.*, Pl. 7, figs. 6 and 7), therefore, almost always takes place on its posterior wall, but I have also come across in sections a certain number of cases where the nephridial ducts of the anterior nephridia cross the dorso-lateral vessel along its anterior wall and then pierce the septum to join the septal excretory canal. Thus we meet with two conditions: either the septum is pierced through before the dorso-lateral vessel is crossed over along its posterior wall, or it is pierced after the vessel has been crossed over along its anterior wall; of these the former is the rule, while the latter is only an exception.

The nephridial ducts from their point of attachment to the septum to the point where they enter the septal excretory canal are 412 μ to 825 μ in length; they become longer as we go to the dorsal side of the animal.

(7) The Ciliation of the Nephridium.—As regards the cilia lining the nephridial tube (Pl. 7, fig. 2), we have to note that they are not distributed uniformly all along its length, but are confined to certain definite regions. I can confidently state that there are at least four ciliated tracts along the course of the nephridial tube. The first tract extends from the funnel to the point where the first free part of the nephridial tube enters the base of the proximal limb of the twisted loop (*a* to *a′* in Pl. 7, fig. 2). Both the second and third ciliated tracts lie in the short straight lobe. As already stated, there are four parallel tubes in this lobe, which go to form the two parallel loops, the outer and the inner (*o.l.* and *i.l.*). There is a ciliated tract in each of the two loops. The second ciliated tract lies in the outer loop, and extends from the apex of this loop to more than half the length of the inner tube of the loop between the letters *bb′*; the cilia in this tract are directed towards the base of the

straight lobe and so work in that direction. The third tract of cilia occurs in the inner loop in its proximal limb along the entire length of the latter between the letters *cc′*. This part of the tube is always brownish and semi-opaque, being full of excretory products; indeed, it is sometimes almost blackish in appearance. We might call the brownish portion the "brown ciliated tube," and note that the cilia in this region work upwards towards the apex of the straight lobe. The fourth ciliated tract lies in the terminal part of the nephridial tube, from the point where it leaves the nephridium to the point where it becomes attached to the septum, between the letters *d* and *d′* (Pl. 7, fig. 2).

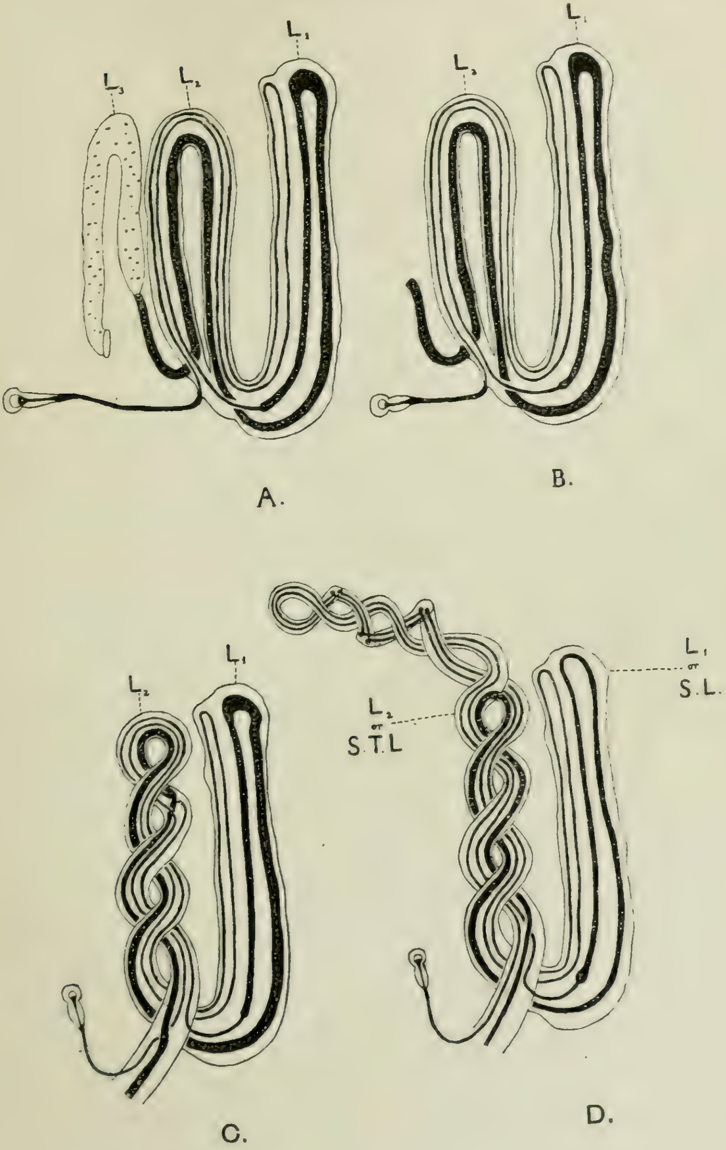
In each ciliated tract the cilia are disposed in a double row, and they move in such a manner as to produce along the axis of the tube a wavy movement closely resembling the flickering of the flagellum or "flame" in a proto-nephridium. (Pl. 7, fig. 2, gives a good idea of both the distribution of the cilia and the flickering movement produced by their action.)

(8) Comparison of the Nephridium of Pheretima with that of Lumbricus.—A comparison between the nephridia of Lumbricus and Pheretima reveals the interesting fact that the latter can be derived from the former by removing its muscular loop and spirally twisting the two limbs of the second loop and then lengthening them out. The accompanying series of diagrams serves to illustrate how the nephridium of Pheretima can be derived from that of Lumbricus.

TEXT-FIG. 3.

A series of four diagrams showing how the nephridium of Pheretima can be derived from the nephridium of Lumbricus. *A* is a diagram of the nephridium of Lumbricus showing the "first" and the "second" loops and the "muscular loop." In *B* the muscular loop is omitted, but the rest is the same as *A*. In *C* the two limbs of the second loop are twisted about each other, forming four twists. In *D* the twisted loop is lengthened out and the resultant is a diagram of a septal nephridium of Pheretima. L_1, the first loop; L_2, the second loop; L_3, the muscular loop; *S.T.L.*, the spirally twisted loop corresponding to the second loop of Lumbricus; *S.L.*, straight lobe corresponding to the first loop in *A*, *B* and *C*.

TEXT-FIG. 3.



A. B.

C. D.

(c) The Ducts and Openings of the Septal Nephridia.

(1) The Septal Excretory Canal (*s.e.c.*, Pl. 6, fig. 1).—The septal canal is a prominent channel running along the posterior face of each septum near its outer border, and, as previously stated, lies internal and parallel to the dorso-lateral vessel along its entire course. There is a pair of these septal excretory canals on each septum—one on each half, right and left. In preparations of the septa in which the outer edge is not injured one can easily see a canal beginning from the ventral side and running internal to and alongside the dorso-lateral vessel; it is generally narrower in diameter than the blood-vessel and has the appearance of being empty or containing a colourless fluid, while the blood-vessel is coloured yellowish owing to the presence of blood; the one can thus be easily distinguished from the other.

The septal canal originates ventrally on each side, right and left, by the union of a few (three or four) of the ducts of nephridia lying in the ventral region. In complete preparations of septa it is easy to demonstrate the commencement of this canal by the union of three or four branches, each of which itself has been formed by the union of several nephridial ducts. The place of commencement of this canal, or rather the place where several branches meet to form this canal, is situated a little higher or dorsal to the place where the dorso-lateral vessel receives a septo-intestinal branch from the intestine (*si.v.*, Pl. 6, fig. 1). The septal excretory canal after its formation runs dorsalwards, as already described, alongside the septal loop, the space separating these two being usually equal to about half the thickness of the septal canal, but not uncommonly the walls of the two are in contact. The septal canal receives the ducts of all the nephridia, anterior and posterior, belonging to its own half of the septum, the nephridial ducts running a fairly long and oblique course before reaching the septal canal, and even after reaching it, many of them continue a short distance alongside it before actually entering. As a rule, each nephridial duct opens separately into the septal canal, but at places one also comes across a nephridial duct emptying its contents into another nephridial duct instead of opening independently into the septal canal. In serial sections (Pl. 7, fig. 6) it is easy to follow a nephridial duct from the place where it leaves the nephridium and runs along the septum to cross the dorso-lateral vessel anteriorly or posteriorly to the place where it enters the septal canal.

On following both the dorso-lateral vessel and the septal excretory canal towards the dorsal side, we find that about the level of the dorsal wall of the gut, they (i.e. the septal canal and the dorso-lateral vessel) begin to diverge from each other; the dorso-lateral vessel bends inwards to join the dorsal vessel, and the septal excretory canal enters the supra-intestinal excretory duct of its own side (Pl. 6, fig. 1).

(2) The Supra-intestinal Excretory Ducts.—These are a pair of longitudinal ducts situated in the mid-dorsal line beneath the dorsal vessel and above the dorsal wall of the gut. They are closely attached to the dorsal wall of the gut, but can be separated by careful dissection. They are always very prominent structures in sections (Pl. 8, figs. 8, 9), and have all along been taken to be blood-vessels. Stephenson (**15**, 1913) mistakes them for a continuation of the supra-intestinal blood-vessel, and says: "The supra-intestinal vessel extends back along the œsophagus and intestine beneath the dorsal vessel, communicating with the hearts in the segments xii and xiii and with the alimentary plexus throughout its extent." At another place he adds: "The supra-intestinal vessel . . . exists only in certain of the anterior (œsophageal) segments of the body in the Tubificidæ; in Pheretima it extends backwards along the intestine also." As a matter of fact, the supra-intestinal vessel does not extend behind the thirteenth segment, since it terminates in that segment by joining the pair of hearts there. In the fourteenth and fifteenth segments there is no blood-vessel nor any other tubular structure, while behind the fifteenth segment we get the supra-

intestinal excretory ducts occupying the same relative position as that occupied by the supra-intestinal vessel in the anterior region. These excretory ducts begin from the septum 15/16 because this is the first septum which bears the septal nephridia. I myself believed these ducts to be blood-vessels for a long time and recorded them as such in my notes and drawings of the blood-system, and it was not until my attention was definitely directed to them by the discovery of their continuity with the septal canals that I recognised their true nature. It need hardly be said that these supra-intestinal ducts are never seen to contain blood, either in dissections or in sections; and they thus offer a striking contrast to the dorsal vessel, which is almost invariably gorged with blood.

The supra-intestinal ducts extend along the entire length of the gut from behind the septum 15/16 to the posterior end of the body of the worm. Anteriorly and posteriorly they become narrow and single, while in the region of the intestine they are always large and paired and, therefore, quite prominent structures. Just a little behind each intersegmental septum these two longitudinal ducts communicate with each other for a very short distance ($30\,\mu$), but otherwise the two are entirely separate although they lie side by side. In sections of a fair-sized worm, these channels in the region of the intestine between two intersegmental septa were about $115\,\mu$ in diameter, while the diameter of the dorsal vessel at that place was about $220\,\mu$. As already mentioned, these supra-intestinal excretory ducts receive a pair of septal excretory canals in the region of each intersegmental septum, so that the excretory fluid from the septal canals is discharged at each intersegmentum into these paired channels, the longitudinal extent of which exactly coincides with the longitudinal distribution of the septal canals and the septal nephridia.

By what means do these supra-intestinal excretory ducts communicate with the exterior for the discharge of their contents? That was a problem which baffled me for many a month, and all attempts at finding an external opening of these longitudinal ducts failed. With this purpose in view I especially examined these ducts at their two ends, anterior and posterior, but with no success, the ducts ending abruptly in those two regions without showing any signs of opening to the exterior. Next, since Beddard (**3**, 1895) has suggested a possible connection between the nephridia and the dorsal pores, I carefully examined my preparations to see if there was any communication between the supra-intestinal ducts and the dorsal pores, but I could find none.

Subsequently, however, while looking at a series of sections of the earthworm in the region of the intestine, I noticed that at one place one of the two supra-intestinal ducts communicated with the lumen of the gut by a distinct opening, and though at the time I concluded that this communication must be accidental in origin, yet it gave me the clue which ultimately led to the correct solution of this problem. Later, following up this clue, I prepared a complete series of sections passing through seven segments of the worm's intestine, and in this series I found exactly seven communications between one or other of the two supra-intestinal ducts and the lumen of the intestine. It thus became certain that these longitudinal excretory ducts did not open at all to the exterior but into the lumen of the intestine, the openings being arranged segmentally. In my serial sections it can be seen that in each septal region one of the two supra-intestinal excretory ducts, the right or the left, takes a dip into the dorsal wall of the gut on one side of the typhlosole, that this dip deepens, and that an actual communication is formed between the supra-intestinal duct and the lumen of the gut by means of a very short and narrow ductule (Pl. 8, figs. 8, 9). This narrow ductule is situated at the rounded corner in the gut where the base of the typhlosole passes into the gut epithelium, or, in other words, where the gut epithelium bends inwards to form the typhlosolar projection into the gut. The narrow ductule has muscular walls when traversing the muscle layers of the gut, but has a special lining of small epithelial cells where it pierces the gut epithelium. The ductule seems to be a laterally compressed structure, elliptical in cross-section rather than circular, since in serial sections of the intestine the ductule measures certainly more than $6\,\mu$ in its longer diameter, as traces of the ductule can be seen both in front of and behind the single section which cuts completely through this ductule and which (the section) itself is $6\,\mu$ in thickness; while when we measure the width of the ductule in this very single section it is about $4{\cdot}2\,\mu$. The ellipsoid ductule, therefore, measures more than $6\,\mu$ in the longer diameter and $4{\cdot}2\,\mu$ in the shorter diameter of its cross-section.

It is remarkable that these narrow ductules leading from the supra-intestinal ducts to the lumen of the gut are, in the first place, segmentally arranged, and in the second place, are so narrow and minute that they can easily be mistaken for cracks in the sections unless they are examined carefully. But in a series of sections passing through five or six segments one never fails to find these narrow ductules in each septal region. It is also interesting to note that if we remove the supra-intestinal ducts from a part of the intestine and examine them after suitable preparation, we are able to see an aperture on one or other of the ducts at each septal plane. These apertures, of course, mark the positions of the downwardly-dipping ductules, which have of necessity been torn off in the process of removal of the supra-intestinal ducts from the intestine.

The supra-intestinal ducts do not both communicate with the gut in each intersegmental place, but it is always only one of them—sometimes the right and sometimes the left—which does so. It might be thought that the right and the left supra-intestinal ducts communicate with the gut one behind the other in a regularly alternate manner, but this is actually not the case. In the series of sections passing through seven intersegmental places the ducts opened into the gut antero-posteriorly in the following order: Left, left, right, left, right, right, right. When either of them has communicated with the lumen of the gut it then communicates with the other supra-intestinal duct for a very short interval ($30\,\mu$ generally), and then the two separate again. At four places I observed these communications; they extended over five sections at each of these four places, each section being 6 μ in thickness, thus giving 30 μ as the width of the communication between the two longitudinal ducts. Since the two ducts communicate with each other soon after one of them has communicated with the gut, the order of opening of the duct of one side or the other into the gut mentioned above is evidently of no consequence.

We must therefore conclude that the excretory products in solution are discharged into the lumen of the gut, whence they are got rid of through the anus along with the fæces.

There are a few points with regard to the septal excretory system (enteronephric) to which I should like to draw attention. The first is its strictly segmental character. It has always been recognised that in earth-worms like Lumbricus the nephridia are segmental in arrangement, and, as a matter of fact, the old name for these structures was "segmental organs"—a term which emphasised their segmental distribution. Although this old name has been given up, still the metameric disposition of the nephridia in Lumbricus and other worms is clearly recognised; but Beddard (**3**, 1895,) writing of the nephridial system of Perichæta (Pheretima) in particular and the plectonephric type of nephridial system in general, says, "The segmentation so clearly visible in most of the organs of the worm's body has here been lost." This assertion, of course, was based on an imperfect knowledge of the nephridia of Pheretima. We now know that the nephridia of Pheretima are clearly segmental structures, since they are serially repeated on each septum behind the fifteenth segment; similarly are the septal canals strictly segmental, as we have a pair of them on each septum. Lastly, the openings of the pair of supra-intestinal ducts are also segmental, since we get one opening into the intestine in each intersegmental space. The whole of the enteronephric nephridial system is, therefore, arranged on a strictly segmental plan.

The multiple character and probable efficiency of these organs is another notable feature. Beddard (**4**, 1910) speaks of the exceeding activity of Perichæta as its distinctive feature, and adds: "Dr. Baird, in a communication to the Zoological Society, remarked upon the agile fashion in which these tropical Annelids will spring off a table when touched or any way interfered with. Gardeners have recognised this character and given it the name of eel-worm." In an active worm of this kind it would be necessary to have a very efficient excretory system. It has been suggested that in a worm in which the cœlomic fluid is constantly flowing from one end of the body to the other and vice versâ, in correspondence with the great activity of the animal, these small nephridia in large numbers, about a hundred or so in each segment, with an equal number of funnels and nephrostomes which would occupy every nook and corner of the segment, would be much more efficient than a single large nephridium with a single nephrostome. There can be no doubt whatever that these nephridia are active excretory organs, and that Perrier's view regarding them as rudimentary is certainly not tenable.

By far the most remarkable feature, however, of the septal nephridial system is the absence of any direct communication between the nephridia and the exterior, as we find in almost all other earth-worms. In Pheretima, on the other hand, we get instead a series of segmentally arranged communications between the nephridial system and the lumen of the intestine. This is entirely a new feature in the morphology of the excretory system of earth-worms, and has been recorded, so far as I know, for the first time in this paper.

(d) The Pharyngeal Nephridia and their Ducts.

The pharyngeal nephridia, as already stated, consist of tufts of these organs surrounding the œsophagus in Pheretima posthuma in the fourth, fifth and sixth segments. In the natural condition these tufts are hidden under the thick septa which separate the fourth, fifth, sixth and seventh segments from one another, and which form a series of cones one inside the other around the œsophagus in these segments; one has, therefore, to remove these septa to a large extent in order to display the pharyngeal nephridia.

(1) Literature.—The pharyngeal nephridia were first described by Beddard in Octochætus. In this genus these nephridia consist of a single pair of tufted organs lying in contact with the pharynx, each tuft possessing a duct which runs forwards and opens into the buccal cavity. These tufted organs were called "salivary glands" by Beddard, and Benham gave them the name of "peptonephridia"—a term accepted by Beddard. Spencer in 1888 (**14**) described in Megascolides australis a large mass of minute tubules occupying most of the space between the pharynx and the body-wall. These tubules were said to be much-coiled structures identical with ordinary nephridia; further, the ducts of these nephridia-like organs appeared to have joined together at intervals to form a common duct, one on each side, and these common ducts opened into the pharynx, their openings having been identified in sections. Michaelson in 1913 (**9**) described a pair of peptonephridia lying lateral to the œsophagus in Periscolex, which were broadly swollen behind and passed in front into a long, narrow duct. Miss Raff (**11**, 1910) says: "A noticeable feature in the dissections of some of the species such as Megascolex dorsalis, Megascolex fielderi and Diporochæta tanjilensis, is the presence of a large number of nephridial tubes attached ventrally in the region of the pharynx. They are in the form of bunches, and have generally been considered as 'peptonephridia.' The latter have been defined by Beddard as nephridia opening into the anterior section of the alimentary canal and functioning in relation to digestion. So far, however, as I have examined, I have found them opening to the exterior, not into the canal; I cannot therefore regard them as peptonephridia, but simply as a specialised group of nephridia, the meaning of which is not clear." Powell (**10**, 1911–12) referred to the pharyngeal

nephridia in Pheretima posthuma, and described them as occurring in the fifth and sixth segments. He seems to have seen the cœlomic funnels of these nephridia, which answer in this and in all other respects to the definition of a nephridium morphologically. He says nothing about the course of the ducts and their openings, and is not certain about the function of these nephridia.

(2) Description of the Pharyngeal Nephridia in Pheretima posthuma.—Beginning from behind, we first come across these pharyngeal nephridia in the sixth segment (*PN_3*, Text-fig. 1). In a dissection from the dorsal side, after removal of the septum 6/7, the pharyngeal nephridia are seen as two large and conspicuous masses of nephridial tubules, one lying on each side of the œsophagus, and resembling, under a lens, a big tuft formed of a large number of strings. On careful examination, after cutting across and reflecting back the part of the œsophagus in the sixth segment, we find that these tufts extend around the œsophagus to the mid-ventral line. Dorsally they cover the greater part of the œsophagus, and also tend to encroach upon the dorsal vessel, which is, however, not covered by them. The paired tufts are thus disposed in two semicircular curves round the œsophagus, each curve extending from about the mid-dorsal to the mid-ventral line. Posteriorly these nephridial masses are loosely attached to the septum 6/7.

Intermingled with the nephridial tufts of this segment are found a large number of rounded cell-masses called the "blood-glands." These rounded structures are not aggregated into follicular spaces here as in the fifth segment (vide infra), but are dispersed among the nephridial tufts. The blood-glands as well as the nephridial tufts have a copious blood-supply, each nephridial tuft receiving a branch from the dorsal vessel, which divides and subdivides to supply blood to the individual elements of these tufts.

The ductules of the individual nephridia join with one another at intervals and form ducts which unite with other similar ducts and so on, and ultimately form a pair of long thick-walled common ducts, one from each nephridial tuft. This pair of common ducts runs forwards, and can easily be seen on each side of the mid-ventral line about 2 to 3 mm. apart in the anterior part of the sixth segment. After traversing the sixth segment these ducts pierce the septa 5/6 and 4/5 in front and open finally into the buccal cavity about the middle of the second segment (*Pd.*$_3$), quite in front of the region of the pharyngeal mass and the supra-pharyngeal ganglion. The openings of these ducts into the buccal cavity have been identified in sections and also verified by a minute dissection of this region.

The nephridial tufts of the fifth segment (*PN.*$_2$) are considerable in size and agree in all essentials with those of the sixth segment. In this section also we have a number of blood-glands; they are, however, not diffuse, but are aggregated into two or more masses in the shape of follicles on each side which resemble bunches of red grapes in the fresh condition. The nephridial tufts here also lie on each side of the œsophagus and extend both dorsally and ventrally; they lie chiefly in the posterior half of this segment, but extend forwards also to cover partially the blood-glands which occupy the anterior half of this segment. It is also seen that some of the nephridia actually penetrate into the blood-glands. Like the nephridial masses of the sixth segment the nephridial tufts here also are connected with a pair of common ducts, one on each side, about 2·5 mm. apart. These ducts run forwards, penetrate through the septum 4/5, and enter the pharynx in the anterior part of the fourth segment, just a little in front of the middle line of setæ of this segment (*Pd.*$_2$).

The third pair of nephridial tufts (*PN.*$_1$) lies in the posterior part of the fourth segment attached to the anterior surface of the septum 4/5. These tufts consist of comparatively fewer nephridia than there are in the fifth and sixth segments. The pair of ducts from these tufts travels only for a short distance forwards, and opens into the pharynx in the posterior half of the fourth segment behind the middle line of setæ (*Pd.*$_1$).

We have thus three pairs of nephridial tufts in the anterior region, a pair in each of the fourth, fifth and sixth segments. We have also three pairs of thick and muscular nephridial ducts, a pair to each pair of tufts, which open into the buccal cavity and the pharynx.

It will be seen that it is a matter of some difficulty to define accurately where the three pairs of ducts from the pharyngeal nephridia open. In a dissection of the first four segments it is difficult to determine the exact boundaries of successive segments, since there are no septa in these segments, the cœlomic cavity being continuous. I therefore made use of serial sections of this region to determine accurately the places of opening of these ducts. In these sections also the dorsal pores marking off one segment from another are absent, and so I had to use the ring of setæ in the middle line of each segment as my criterion for determining the boundaries of successive segments.

As regards the structure of each pharyngeal nephridium, we have to note that it is very similar to that of a septal nephridium. The pharyngeal nephridium also consists of a short straight lobe and a long spirally twisted loop, the proximal limb of the latter being continued out as the terminal nephridial duct. But I have never been able to see the "funnel" in these pharyngeal nephridia, in spite of repeated attempts, and have come to believe that it is absent. Powell (**10**, 1911–12), however, writing about these nephridial nephridia of Pheretima posthuma, says: "Under the microscope and with the aid of sections each of these strings is seen to be a tubular structure opening into the cœlom by a slightly funnel-shaped aperture bearing a ciliated epithelium." As I have already stated, I have not been able to see the "slightly funnel-shaped apertures" of these nephridia, and cannot therefore corroborate Powell's statement. In the short straight lobe the second and third ciliated tracts can easily be made out, and work in the same way and in the same direction as a septal nephridium; but I have not been able to make out the first and fourth ciliated tracts here. Whether these nephridia are excretory in function or have a peptic function in connection with assimilation is still to my mind problematical.

(e) The Integumentary Nephridia.

The integumentary nephridia (*i.n.*, Text-fig. 1) are attached in Pheretima to the inner surface of the integument, or, in other words, to the somatic layer of the peritoneal lining of the cœlomic cavity. They are absent in the first two segments of the worm, but are present in all the succeeding segments. In each segment they extend all along the surface of the body-wall from the mid-ventral to the mid-dorsal line and are not arranged in any definite order, but are scattered promiscuously. It is seen, however, that they are more abundant midway in each segment on both sides of the line of setæ and are more sparse at the two ends of each segment, i.e. near the septa. They are best seen in specimens which have been fixed in acetic bichromate solution after they have been opened by a mid-dorsal incision and the flaps of skin pinned down.

The nephridia are extremely minute structures, hardly visible to the naked eye. They are attached to the body-wall only by means of their terminal ducts, i.e. in the same way as the septal nephridia are attached to the septa. The lobes and loops of these nephridia project out into the cœlomic cavity from the inner surface of the body-wall as V-shaped structures, the two limbs of the V being unequal in size and representing the straight lobe and the twisted loop of the septal nephridia (Pl. 7, fig. 7).

The number of these nephridia seems to vary in different segments. In those segments which possess septal nephridia the number of these integumentary nephridia is not very large; on counting I found about 250 nephridia in the thirtieth and about 200 in the thirty-ninth segment. But in the segments 14, 15 and 16, where the septal nephridia do not exist or only begin, and which are also incidentally the clitellar segments, there is, so to speak, a "forest" of these integumentary nephridia, these being so numerous and so closely set as to cause the inner surface of the body-wall in these segments to look, under a lens, as if it were covered with dense fur (*F.I.N.*, Text-fig. 1). They are more than ten times as numerous on these three segments as on the preceding or succeeding segments; I was able to count as many as 220 on a piece of skin 2 mm. square from the fourteenth segment.

Like the septal nephridia, these integumentary nephridia also are separate from each other; each nephridium is a discrete structure and opens separately by its duct to the exterior on the skin (Pl. 7, fig. 7). There is no network of any kind formed by the tubules of separate nephridia, nor is there a connection between nephridia of successive segments by means of tubules perforating the septa, as suggested by Beddard (**2**, 1888). The number of nephridiopores on a segment will equal the number of nephridia in that segment. Beddard (**2**, 1888) states that the number of nephridiopores is greater anteriorly than posteriorly; it is very probable that he counted the nephridiopores on one of the three segments (fourteenth, fifteenth and sixteenth) which we have referred to above as possessing a "forest" of nephridia, and compared this number with the number of nephridiopores on any of the succeeding segments, and this led him to make the above-mentioned statement.

These nephridia are, as already mentioned, very minute; each is less than half the size of a septal nephridium; a nephridium of an average size when measured has the straight lobe about 100 μ in length and the long twisted loop about 182 μ in length. I have not been able to find any funnels in connection with these integumentary nephridia, but otherwise they are like the septal nephridia in their general structure.

(f) The Significance of the "Enteronephric" Type of Nephridial System.[1]

Benham (**4**, 1910), describing the excretory functions of the nephridia in Polychætes, writes: "Excretion, in the strict sense of the word, is carried out by the cells forming the wall of the nephridial tube; they remove waste materials from the blood distributed over the surface of the organ. But, in addition, there is a removal from the cœlom, by means of the funnel, of any dead or dying cœlomic corpuscles, which in their turn have eaten up or otherwise destroyed foreign bodies (such as Bacteria, etc.) that may have entered the animal." In Pheretima also, the septal nephridia have a copious blood-supply and possess cœlomic funnels with nephrostomes; we may therefore safely assume that here also as in Polychætes the nephridia discharge the function of excreting waste materials both from the blood and the cœlomic fluid. In the case of other worms the nephridia open to the exterior and all the excretory products are got rid of through the nephridiopores on the skin. But what can be the meaning of the elaborate "enteronephric" type of nephridial system in Pheretima, in which the excretory ducts ultimately open into the lumen of the alimentary canal along almost its entire length and presumably discharge all the excretory products into the gut?

The previously recorded cases of nephridia opening into the front and hind ends of the gut have been explained, on the one hand, as being peptic in function (peptonephridia),

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[1] [Mr. Karm Narayan Bahl has not entered upon any discussion concerning the significance of his remarkable discovery of "enteronephric" nephridia in relation to the commonly-accepted view that all Annelid nephridia are ectodermal. But it seems to be evident that the septal nephridia of Pheretima certainly cannot be developed from the ectoderm but must be mesodermal in origin, since we can hardly suppose that they are endodermal outgrowths. Mr. Karm Narayan, however, has recently informed me that he has been successful in collecting a large number of Pheretima embryos and hopes to be able to ascertain the exact mode of development of the septal and other nephridia.—W. N. F. WOODLAND.]

and compared, on the other, with the respiratory trees of Bonellia and its allies and with the Malpighian tubes of the Arthropoda (Beddard, **3**, 1895). Evidently we cannot explain away the segmentally arranged openings of this "enteronephric" system into the lumen of the gut in either of these two ways. These segmental openings must have a deeper significance. It has been suggested to me by Dr. Woodland that this enteronephric system is probably an adaptation for conservation of water in a tropical worm like Pheretima. This genus is Oriental as well as Australian in distribution, but is rare in the Australian region. There is no doubt that these worms have to adapt themselves to a very wide variation in the conditions of moisture: the region they inhabit is subject to a prolonged period of drought alternating with a short period of abundant moisture during the monsoon rains. Normally there are three to four rainy months alternating with eight or nine dry months in a year.

On à priori grounds, we must conclude that the excretory matters removed from the blood and the cœlomic fluid pass in a state of solution into the lumen of the gut viâ the system of ducts connected with the septal nephridia. In the intestine, we must assume that the water containing excretory matter in solution is re-absorbed by the gut epithelium for the needs of the organism, while the solid excretory matters pass out of the body through the anus. We are thus crediting the gut epithelium with the power of separating water from the excretory matters dissolved in it and then re-absorbing this water. Although there is, as yet, no experimental evidence in favour of this hypothesis, still we might mention one or two points in regard to this hypothesis very briefly. It is stated by Thomson (**17**, 1906) that when an earthworm has been made to eat powdered carmine, the passage of these useless particles from the gut to the yellow cells, from the yellow cells to the body-cavity and thence out by the excretory tubes can be traced. If that be so, we must conclude that the gut epithelium of the earthworm takes up these carmine particles and passes them on to the yellow cells

—or in other words, the gut epithelium acts as an organ of excretion in removing the useless carmine particles from the intestine; this, again, is a commonly admitted property of the intestinal epithelium. But we have to go further and ask how it is that, while the intestinal epithelium absorbs the carmine particles, it does not also absorb the excretory matter which is poured into the intestinal lumen viâ the excretory ducts? Why is it that from these it takes up only water and lets go the solid waste matter? In order to get over this difficulty we must assume that, like the intestinal epithelial cells of man, these cells of the earthworm also exercise a sort of protective function dependent on that capacity for physiological selection by which the epithelia of the intestine, while they absorb certain substances, do not permit others to pass into them.

Another consideration is about the relative importance of the septal and the integumentary nephridia. The latter are small in size but enormous in numbers, and open directly to the exterior through the skin. But as these nephridia do not possess any funnels and are closed internally, we should conclude that water cannot pass out in any quantity through them, although it may diffuse though the walls of the nephridia. There is very little loss, therefore, through these integumentary nephridia, and we may presume that all water is re-absorbed and conserved by the worm.

4. The Nephridial System of some other Species of Pheretima.

Besides Pheretima posthuma, the nephridial system of which I have described in detail above, I have examined the nephridial systems of three other species of the genus Pheretima, namely, P. hawayana, P. heterochæta and P. barbadensis. In general outline the nephridial system in these three species of Pheretima is similar to that in Pheretima posthuma, but there are certain differences which I shall mention below.

(1) Pheretima hawayana.—This species also possesses

the three kinds of nephridia, namely, the septal, the pharyngeal and the integumentary. These nephridia are very similar in position and structure to those of P. posthuma. We may only note that the septal nephridia here are larger than those of P. posthuma; the funnel is identical in structure with that of the latter species, but the first part of the nephridial tube, which makes a bend before joining the main body of the nephridium, is much longer in this species than in P. posthuma; in the latter it measures, on an average, about 84 μ in length, while in P. hawayana it is generally more than 180 μ long.

The septal excretory canal running parallel to the dorso-lateral vessel does not lie near or in contact with the vessel as in P. posthuma, but is removed a good distance from it: I measured the distance to be about 165 μ in one and about 412 μ in another specimen. As a consequence of these two structures lying far apart from each other, the terminal nephridial ducts have a very much longer course to run on the septum to meet the septal canal than they have in P. posthuma. Moreover, as the dorso-lateral vessel is not attached to the septum as in P. posthuma, the nephridial ducts do not cross this blood-vessel at all. We may also note in passing that the septa of P. hawayana are much more muscular than the septa of P. posthuma, and therefore in the former the septal canal and the terminal nephridial ducts on the septum are obscured by the muscle-fibres, and it is only by a careful examination that these structures can be made out.

The integumentary and the pharyngeal nephridia are identical in structure with those in P. posthuma.

(2) Pheretima heterochæta and Pheretima barbadensis.—The nephridial system in these two species resembles very closely that of P. hawayana rather than that of P. posthuma; the septal excretory canal is separated a good distance from the dorso-lateral vessel and the free duct following the funnel is much longer than that in P. posthuma.

5. THE BLOOD-SUPPLY OF THE NEPHRIDIA IN PHERETIMA.

Benham (**6**, 1891) has described in detail the vascular supply of the nephridium of Lumbricus, and in general outline this description applies to the vascular supply of the nephridia of Pheretima, but there are a number of interesting points in which the blood-supply of the nephridia in the two cases differs, these differences being due, firstly, to the much larger number of nephridia present in Pheretima, and secondly, to the fact that the nephridia of Pheretima are of three different kinds situated in three different regions of the body.

As in Lumbricus, the ventral vessel in Pheretima gives off, in the posterior part of each segment, a vessel on each side, which we may call the parietal vessel (*p.v.*, Pl. 6, fig. 11). This vessel, soon after its origin, perforates the septum behind and runs along the middle line of the inside of the body-wall of the succeeding segment ventro-dorsally (e.g. the ventral vessel in the twentieth segment gives off a pair of parietal vessels which perforate the septum 20/21 on each side and distribute blood over the inside of the body-wall of the twenty-first segment). The parietal vessel as it perforates the septum gives off a septal branch (*s.b.*), which runs along the septum very near its attachment to the body-wall parallel and external to the dorso-lateral vessel. This last vessel, as shown in the diagram, is a commissural vessel in each segment, joining the subneural with the dorsal vessel (*dl.v.*, Pl. 6, figs. 1 and 11). I may add, in passing, that there is no direct connection between the ventral and subneural vessels as has been supposed by some authors to be the case.

Although it is a difficult matter to decide what course is taken by the blood outside the large median trunks, yet from a comparison with the course of blood in Lumbricus and also from a number of observations on the distribution of valves in the blood-vessels, I am led to believe that the blood passes to the nephridia from the ventral vessel, and that it is brought back to the dorsal vessel from the nephridia by way of the dorso-lateral vessel and the subneural vessel. The parietal vessel, as mentioned above, runs along the middle line of the inside of the body-wall ventro-dorsally, and during its course gives off a number of branches which ramify over the area of the body-wall of the whole segment, the vessel terminating near the mid-dorsal line. Now the whole of the inside of the body-wall is covered over by a large number of integumentary nephridia, and thus these are supplied with blood from the branches of the parietal vessel which also supplies blood to the body-wall. We have already said that the parietal vessel gives off a septal branch (*s.b.*, Pl. 6, fig. 11) as it perforates the septum. This septal branch is rather difficult to make out in preparations of the septa, since it lies very near the outer edge of the septum, in which position it is very generally torn off in the removal of the septum; its origin from the parietal vessel and its extent along the septum can both be seen, however, in preparations in which the outer edge of the septum is intact and the parietal vessel perforating the septum is also included. The septal branch runs parallel to the dorso-lateral vessel and is about one-third the diameter of the latter. All the septal nephridia receive their blood-supply from the septal branch, which throughout its entire course gives off a large number of twigs (*tt*), each twig supplying a septal nephridium. The blood from each septal nephridium is returned by another minute vessel (*t′t′*) which runs parallel to the first in the base of the nephridium, and which joins, along with others of its kind, the dorso-lateral vessel. Each septal nephridium (Pl. 6, fig. 12), therefore, has two vessels in its basal part; the first one arises from the septal branch and enters and brings blood to the nephridium (we might call this the afferent nephridial vessel, *tt*); the second brings back blood from the nephridium and leaves the nephridium to join the dorso-lateral vessel into which the blood is now poured (we might call this vessel the efferent nephridial vessel, *t′t′*). In each septal nephridium both the afferent and efferent vessels fork near the base of the nephridium, one branch of each fork entering the straight lobe and the other branch entering the twisted loop (Pl. 6, fig. 12). These branches are continuous at their distal ends (*dd*), and there are also cross connections between them during their course (*cc*). We may note that the afferent vessel and the two branches of its fork are generally empty or contain very little blood, while the efferent vessel and the branches of its fork are full of blood; similarly the septal branch contains very little blood, while its counterpart, the dorso-lateral vessel, is generally full; this phenomenon corresponds with the condition of arteries and veins found after death in the higher animals, and lends additional support to the assumption made that the septal branch and the afferent vessels carry blood to the nephridium, while the efferent vessel removes the blood from it and pours it into the dorso-lateral vessel. The dorso-lateral vessel also receives a large number of branches from the body-wall, which presumably bring back blood from the body-wall proper and also from the integumentary nephridia. Another way by which blood from the integumentary nephridia may be returned to the general blood-stream is by means of capillaries and twigs that join together to enter the subneural vessel in each segment. The septal branch also receives small branches from the skin which possibly replenish its supply of blood for the septal nephridia. To sum up, the parietal vessels supply blood to the integumentary nephridia and body-wall directly, and to the septal nephridia through their septal branches, while the blood is returned from the integumentary and septal nephridia to the dorso-lateral vessel and possibly to the subneural vessel also (to this only from the integumentary nephridia).

In the case of the pharyngeal nephridia the blood-supply is different. In the fourth, fifth and sixth segments the dorsal vessel gives off in each segment a pair of branches, the branch of each side supplying the pharyngeal nephridia and the septa. In a freshly-killed worm in which the dorsal vessel is still pulsating, one can easily see the flow of blood from the dorsal vessel to the pharyngeal nephridia through the branches referred to above. The blood is collected from these pharyngeal tufts of nephridia by capillaries, which unite together and open ultimately into the lateral œsophageal vessels which are situated ventro-laterally to the œsophagus, one on each side, and from which the blood goes into the blood-stream viâ the supra-intestinal vessel and the subneural. When a worm is opened some time after its death, it is always seen that the branches from the dorsal vessel are empty, while the branches carrying blood to the lateral œsophageal vessels and the latter themselves are full of blood.

6. The Intersegmental Septa in Earthworms.

The intersegmental septa of earthworms have always been regarded as partitions, partly membranous and partly muscular, which stretch across from wall to wall of the body and correspond roughly in their position to the grooves which divide the body externally into somites. The septa are perforated by the gut, the dorsal and the ventral vessels and also by the nerve-cord; the partitioning of the cœlom is incomplete around these structures in most earthworms, and there is free communication from one end of the body to the other for the cœlomic fluid. Except for holes and slits round the nerve-cord and other structures mentioned above, there is no record of any definite apertures, so far as I know, on the septa of earthworms. As a matter of fact, very little interest seems to have attached to the structure and disposition of septa as they had nothing peculiar about them, and they are dismissed in descriptions usually in a line or two. An interesting feature about these intersegmental septa presents itself, however, in Pheretima posthuma and other species of the same genus, which has been described here, I believe, for the first time: it is the presence of a large number of definite apertures each with a sphincter round it. In the following paragraphs I shall provide a description of the septa in Pheretima posthuma, together with an account of these sphinctered apertures, and then compare these features with those of other species and genera that I have examined, and lastly, I shall discuss the possible significance of these sphinctered apertures in the septa of Pheretima.

(1) The Intersegmental Septa in Pheretima posthuma.—Beginning from the anterior end we find that the first definite septum lies between the fourth and fifth segments, the body-cavity of the first four segments being undivided or continuous, although it is traversed by a number of muscular strands passing from the buccal cavity and the pharynx to the body-wall. This first septum is thin and membranous, but the following five septa are very thick and muscular and resemble the intersegmental septa of Lumbricus. These separate the segments 5 and 6, 6 and 7, 7 and 8, 8 and 9 or 9 and 10, and 10 and 11 from each other; between the eighth and tenth segments there is always one septum; sometimes there is one between the eighth and ninth segments and septum 9/10 is missing, and vice versâ; both these septa are never present in the same worm. It happens that the lines of attachment of these septa on the alimentary canal are considerably behind (posterior to) their lines of attachment on the body-wall, and therefore these septa are not transverse in position, but form five cones, one inside the other, with their apices directed posteriorly (Text-fig. 1). These septa are incomplete over the ventral nerve-cord which perforates them, but besides these perforations and those of the gut and blood-vessels, these septa are quite complete partitions with no apertures on them. They are very thick and are formed of muscle-fibres disposed obliquely in two sheets, and have muscular strands passing from their anterior and posterior surfaces to the body-wall or to other septa in order to support them and keep them in position.

Behind the first eleven segments in which we have the specially thick septa mentioned above there is a regular series of thin and membranous septa separating successive segments from each other interiorly. The septa 11/12, 12/13 and 13/14 are complete like the first six, although thin and membranous, the last two being intimately attached to the walls of the "hearts" in the twelfth and thirteenth segments. The next septum, i.e. 14/15, however, is typical of all the septa that follow, and besides being thin and membranous in texture and incomplete over the nerve-cord like the preceding septa, it is specially characterised by the fact that it is riddled with holes over the greater part of its area. These holes or apertures are either oval or circular in outline and are scattered over the lateral parts of each septum, being absent from the dorsal and ventral regions and also from the inner and outer edges. Each of these apertures possesses a thick sphincter around it formed of non-striped muscle-fibres arranged in a concentric manner (Pl. 8, fig. 13).

These sphinctered apertures of the septa vary considerably in size; in my preparations I have found them to measure from 8 μ to 1650 μ in diameter. Very likely the apertures with a small diameter are in a closed condition, while those with a larger diameter are open. The thickness of the sphincters varies from 9 μ to 28 μ; it is, as would be expected, greater in closed apertures than in open ones. As regards the number of these apertures on the septa, I counted as many as sixty-eight on the left half of the septum 15/16, including one of the largest diameter I have come across (1650 μ). The number of apertures on the septa diminishes towards the posterior end of the worm.

The incompleteness of the septa round the nerve-cord is formed by a large circular hole in each septum which surrounds the nerve-cord, and the wall of which is made up of a thick, strong sphincter, the ventral part of which is attached to the body-wall. It may be noted that besides this large hole round the nerve-cord there is no slit or hole round the other structures that pierce the septa, e.g. the gut and the blood-vessels, in Pheretima, the septa round these latter structures being quite complete in this genus.

(2) Septa in Other Earthworms.—The septa in the

other species of Pheretima that I have examined, i.e. P. hawayana, P. heterochæta and P. barbadensis, have much more of muscular tissue in them than the septa of Pheretima posthuma. Moreover, although the sphinctered apertures are present on the septa of these species, the number of these apertures is very small indeed as compared with those in P. posthuma. I have never seen more than one or two on each septum, though they were always of a large size. I examined specimens of several other genera of earthworms in order to see if the septa in them had sphinctered apertures, but could not find any. They are absent in the septa of Megascolex, Perionyx, Lumbricus and Eutyphœus—the genera I have examined for this purpose. It is thus evident that, so far as is known at present, the sphinctered apertures characterise the septa of Pheretima only.

(3) The Significance of the Sphinctered Apertures.—The sphinctered apertures, together with the incompleteness of septa around the nerve-cord, are apparently the means by which the cœlomic fluid is allowed to pass from one segmental chamber into another. It is quite easy to see the flow of the cœlomic fluid inside the body-cavity in a living worm which happens to be a little more transparent than others. The sphincter muscles round these apertures are à priori the means of closing these apertures and thus restricting the flow of the cœlomic fluid to particular segmental chambers, and this isolation of these particular segments would be complete so far as the flow of the cœlomic fluid is concerned when the sphincter round the nerve-cord also contracts and closes that passage. This restriction of the cœlomic fluid to particular segments would lead to a condition of turgidity in those segments and make the worm stiff. It has been suggested that this stiffness would be necessary for the worm in its ordinary locomotory movements either on the surface of the soil or in burrowing, since, for the leverage of a particular part of the body to the ground by means of setæ (the organs of locomotion) it would be necessary

to have that part turgid and stiff, as the setæ on a limp part would not be able to secure a firm hold on the ground. The situation of the setæ in the middle of each segment in the form of a ring is most appropriate for balancing, and I agree with Beddard (**5**, 1912) in thinking that "the ring of setæ would seem to be a character specially suited to an underground life, where there is an equal pressure all round the body, and where progression would seem, therefore, to be best attained by a continual leverage round the circular body."

But why the sphinctered apertures should be present only in Pheretima and not in Megascolex and Perionyx, which are also forms with perichætine setæ, I cannot say.

7. Summary.

(1) There are three distinct kinds of nephridia in Pheretima posthuma and other species of the genus, namely, the septal, the pharyngeal and the integumentary, named according to the position they occupy in the worm; they differ from each other in size and also in respect of the place of opening of their ducts.

(2) Each nephridium is a separate and discrete structure and there is no network of any kind, and therefore the terms "plectonephric" and "diffuse" are inapplicable to these nephridia, although the term "micronephridia" can be retained.

(3) The integumentary nephridia are very minute and are hardly visible to the naked eye. Each of them has its own separate duct opening separately to the exterior on the skin. Each segment has about 200 to 250 of them. The pharyngeal nephridia occur in paired tufts lying at the sides of the œsophagus in the fourth, fifth and sixth segments; these nephridial tufts have three pairs of ducts, one in each segment, which open into the buccal cavity and the pharynx in the second and fourth segments.

(4) The septal nephridia are attached to both sides of the

septa; they do not open on the skin, but are connected with an elaborate system of ducts which ultimately open into the lumen of the intestine mid-dorsally at segmental intervals. The system of ducts and openings is perfectly segmental in arrangement, and consists of a pair of septal excretory canals on each septum which open into a pair of supra-intestinal excretory ducts lying in the mid-dorsal line of the worm above the dorsal wall of the gut, these ducts communicating with the lumen of the intestine at each intersegmental place by means of a narrow ductule. This last feature is characteristic of this type of nephridial system, and the latter has therefore been termed the "enteronephric" type of nephridial system.

Each septal nephridium has a remarkable feature in that it lies—the funnel and all—wholly within the bounds of a single segment. The septal canals, the supra-intestinal ducts and their openings into the lumen of the gut have been described here for the first time, and the segmental character of the nephridial system of Pheretima, which was denied by Beddard, has now been established.

(5) The enteronephric nephridial system is probably a device for the conservation of water in this tropical genus, the gut-epithelium reabsorbing the water of the excretory fluid while letting the solid excretory matters pass out through the anus along with the fæces.

(6) Each nephridium in Pheretima, like that in Lumbricus, is connected with two blood-vessels, one bringing blood to it and the other taking away the blood. The septal and the integumentary nephridia are supplied with blood from the ventral vessel through the parietal vessel and the septal branch, and the blood is returned from these nephridia to the general blood-stream viá the dorso-lateral vessel and the subneural vessel. The pharyngeal nephridia receive their blood-supply from the dorsal vessel, and the blood is returned from them to the lateral œsophageal vessels.

(7) The intersegmental septa in Pheretima posthuma and other species have definite, circular or oval apertures,

each with a sphincter muscle around it. It is suggested that by closing these apertures by means of sphincters there is a restriction of the cœlomic fluid to certain segments which become consequently turgid and stiff, and are thus able to help the setæ in having a firm hold of the ground during the locomotory movements of the animal; this turgidity of the part of the body applying setæ on to the ground seems necessary, since a limp part cannot fix its setæ on the substratum on which the worm moves.

8. Technique and Material.

Worms both for dissection and section-cutting were narcotised in weak alcohol (10 per cent. to 15 per cent.) for fifteen minutes or thereabouts. This method is much more useful than narcotising with chloroform, since the worms with this method do not contract at all, but die in a quite flaccid condition, and can easily be stretched to the utmost. The method of fixation most successfully employed for nephridia of all the three kinds as well as the intersegmental septa was immersion in acetic bichromate (3 per cent. potassium bichromate added to 5 per cent. glacial acetic acid) for forty-eight hours or more. Before fixing, however, the nephridia were fully exposed by opening the worms and pinning down the flaps of skin; a further precaution was found necessary in the fixation of these nephridia, and that was to wash off all cœlomic fluid before putting the worms in the fixative, as otherwise the cœlomic fluid coagulates near the nephridia and renders the fixation of the latter imperfect. In order to get the septa fully stretched they should be exposed by making a mid-dorsal incision in the worm, and pinning the flaps of the skin at intervals of five or six segments in such a way as to keep the intermediate four or five septa on stretch, but not to tear them at all. In this stretched condition the septa should be kept in the fixative for a week or two, after which time they become quite hardened and crisp, and can be removed very easily from their attachment with the body-wall and the wall of the gut. Metallic dishes should not be used for preserving these various structures in bichromate; glass dishes or enamelled pie-dishes with the bottom filled up with hard paraffin answer the purpose very well.

For sectioning worms were fed on moist blotting-paper for a fortnight or thereabouts, after which time the gut is quite free of earth, and can be sectioned without injuring the razor. The sections were stained in the usual way with Delafield's hæmatoxylin, differentiated with acidulated water as recommended by Thapar (**16**, 1918), and "blued" with ammonia vapour after dehydration and clearing. For staining individual nephridia, I found a very weak solution of Delafield's hæmatoxylin followed by acidulated water very useful—even better than borax-carmine and acid alcohol; these hæmatoxylin preparations were very helpful in the investigation of the structure of the funnel, and specially the exact course of the nephridial tube in the body of the nephridium.

For a demonstration of the long terminal nephridial ducts and their openings into the septal excretory canals as well as of the commencement and extent of the latter, preparations stained with either borax-carmine or hæmatoxylin were made of the entire septa, and also a series of longitudinal sections was taken through six segments of the body-wall to which septa were still attached. In these sections I could follow the nephridial ducts from the place where they leave the nephridia to the point where they enter the septal excretory canal. I also incidentally came across many longitudinal sections of funnels in these preparations which were very helpful in ascertaining their structure.

The connections of the septal canals with the supra-intestinal excretory ducts were seen in preparations of the upper parts of the septa together with the supra-intestinal ducts, the last having been dissected out under a Zeiss binocular microscope. Similarly the ducts of the pharyngeal and the septal and integumentary nephridia themselves were also dissected under the binocular microscope, which was very helpful all along.

The segmental openings of the supra-intestinal excretory ducts into the lumen of the intestine were identified in serial sections 6 μ in thickness passing through seven segments of the intestine.

Besides Pheretima posthuma, the species common in Allahabad, I have also examined three other species of Pheretima, namely, P. heterochæta (Mchlsn.), P. hawayana (Rosa) and P. barbadensis, specimens of which were kindly sent to me by Col. Stephenson from Lahore. Specimens of Lumbricus, Perionyx, Eutyphœus and Megascolex were also examined for their septa.

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EXPLANATION OF PLATES 6, 7 AND 8.

Illustrating Mr. K. N. Bahl's paper "On a New Type of Nephridia found in Indian Earthworms of the Genus Pheretima."

Fig. 1 (Pl. 6).—A diagrammatic representation of the septal nephridial system and its relative position in Pheretima posthuma. Three segments are shown, and part of the skin on the left side of the posterior (third) segment has been cut and reflected forwards so as to lie on the second segment, in order to expose the various structures. *b.w.*, body-wall; *b.w.*, body-wall cut and reflected forwards; *i.s.*, intersegmental septum; *s.a.*, sphinctered apertures; *d.v.*, dorsal vessel; *si.e.d.*, supra-intestinal excretory ducts; *g.*, gut; *ty.*, typhlosole; *v.v.*, ventral vessel; *sn.v.*, subneural vessel; *dl.v.* dorso-lateral vessel or the "septal loop"; *s.e.c.*, septal excretory canal; *t.n.d.*, terminal nephridial ducts; *s.n.*, septal nephridia; *si.v.*, septo-intestinal vessel. × cir. 17.

Fig. 2 (Pl. 7).—A septal nephridium showing the course of the nephridial tubule and its "ciliated tracts." *a–a'*, the first ciliated tract; *b–b'*, the second, *c–c'*, the third, *d–d'*, the fourth ciliated tract; *x*, the place of extrance and exit of the nephridial tube; *b.c.t.*, the "brown ciliated tube"; *o.l.*, the outer loop and *i.l.* the inner loop in the straight lobe; *p.l.*, the proximal limb, and *d.l.* the distal limb of the twisted loop; *f.*, funnel. × 220.

Figs. 3 to 7 inclusive are given in Pl. 7.

Fig. 3.—The funnel of a septal nephridium from the ventral side. *u.l.*, upper lip formed of the *m.c.*, marginal cells; *l.l.* lower lip; *n.*, nephrostome; *f.n.t.*, the free first part of the nephridial tube with intra-cellular ciliated lumen; *c.e.c.*, cœlomic epithelial cells. × 800.

Fig. 4.—The funnel in longitudinal section. *c.c.*, central cell; *m.c.*, marginal cell with cilia presenting a bushy appearance: other letters as above. × 800.

Fig. 5.—The funnel from the dorsal side. All the letters as above. × 800.

Fig. 6.—*A*, *B*, *C* and *D*. A series of longitudinal sections of the body-wall with the septa and septal nephridia attached, showing the nephridial ducts crossing over the dorso-lateral vessel to enter the septal excretory canal. 1, 2, 3, 4, 5, 6, the nephridial ducts in sections; *s.e.c.*, the septal excretory canal; *dl.v.*, dorso-lateral vessel; *nph.*, septal nephridia cut in various ways in sections; *b.w.*, body-wall; in *B* the nephridial duct No. 1 is entering the septal canal; in *C* the duct No. 2 is beginning to enter, and in *D* it has been absorbed into the septal canal; other ducts are seen to be travelling towards and approaching the septal canal. × 125.

Fig. 7.—A diagrammatic representation of a part of the body-wall with the septum attached, showing the dorso-lateral vessel and the septal excretory canal. Two septal nephridia and several integumentary ones are also shown. One of the latter is opening to the exterior on the body-wall. *s.s.*, setal sacs; *i.n.*, integumentary nephridia; all other letters as in fig. 1, Pl. 6.

Fig. 7A is a diagram in outline of fig. 7 to show the plane of the sections (three indicated in the diagram) cut, portions of which sections show the structures depicted in fig. 6.

Figs. 8 to 10 inclusive are given in Pl. 8.

Fig. 8.—A section passing through the dorsal part of the intestine in the region of the intersegmental septum and the lymph-gland, showing the left supra-intestinal excretory duct communicating with the lumen of the intestine on one side of the typhlosole. *si.e.d.*, supra-intestinal excretory ducts, the one on the left opening into the gut by a slit *o.*; *g.* gut; *l. g.*, lymph-gland; *ty.*, typhlosole. × 94.

Fig. 9.—Part of fig. 8 highly magnified. All letters as above. × 272.

Fig. 10.—A transverse section of the earthworm Pheretima posthuma passing through the fourth segment, showing the three pairs of ducts of the pharyngeal nephridia in section, $p.d._1$, $p.d._2$,

p.d.$_3$; on the left side, the duct (*p.d.*$_1$) of the pharyngeal nephridia of the fourth segment is entering the pharynx (the other ducts enter in the anterior sections). *b.w.*, body-wall; *p.h.*, pharynx; *ph.s.*, pharyngeal shelf; *ph.m.*, supra-pharyngeal mass; *d.v.*, dorsal vessel; *n.c.*, nerve-cord; *v.v.*, ventral vessel; *p.d.*$_1$, duct of the pharyngeal nephridia of the fourth segment, that of the left side entering the lumen of the pharynx; *p.d.*$_2$, duct of the pharyngeal nephridia of the fifth segment; *p.d.*$_3$, duct of the pharyngeal nephridia of the sixth segment. × 120.

Fig. 11 (Pl. 6).—A diagrammatic representation of the blood-supply of the septal and the integumentary nephridia. *v.v.*, ventral vessel; *n.c.*, nerve-cord; *sn.v.*, subneural vessel; *si.v.*, septo-intestinal vessel; *p.v.*, parietal vessel; *dl.v.*, dorso-lateral vessel; *s.b.*, septal branch; *tt.*, afferent nephridial vessel; *t′t′.*, efferent nephridial vessel; *s.n.*, septal nephridia; *i.n.*, integumentary nephridia.

Fig. 12 (Pl. 6).—A septal nephridium of Pheretima posthuma, showing the course of the blood-vessels in it. *s.b.*, septal branch; *dl.v.*, dorso-lateral vessel; *tt.*, the afferent nephridial vessel; *t′t′.*, the efferent nephridial vessel; *dd.*, the top end of the forks where the afferent vessel passes into the efferent vessel; *cc.*, cross-branches connecting the afferent vessel with the efferent vessel. × 220.

Fig. 13 (Pl. 8).—Portion of an intersegmental septum showing the sphinctered apertures in Pheretima posthuma. *s.a.*, sphinctered apertures; *m.f.*, muscle-fibres traversing the septum. × 165.

N.B.—Pl. 6 contains figs. 1, 11 and 12. Pl. 7 contains figs. 2, 3, 4, 5, 6, 7 and 7A. Pl. 8 contains figs. 8, 9, 10 and 13.

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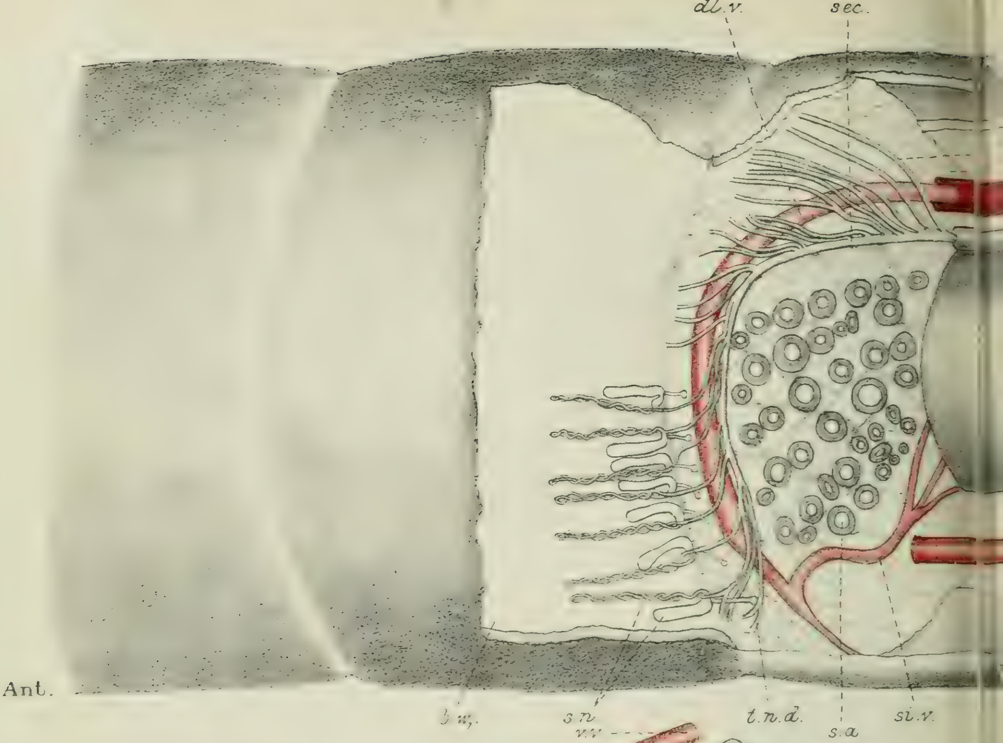
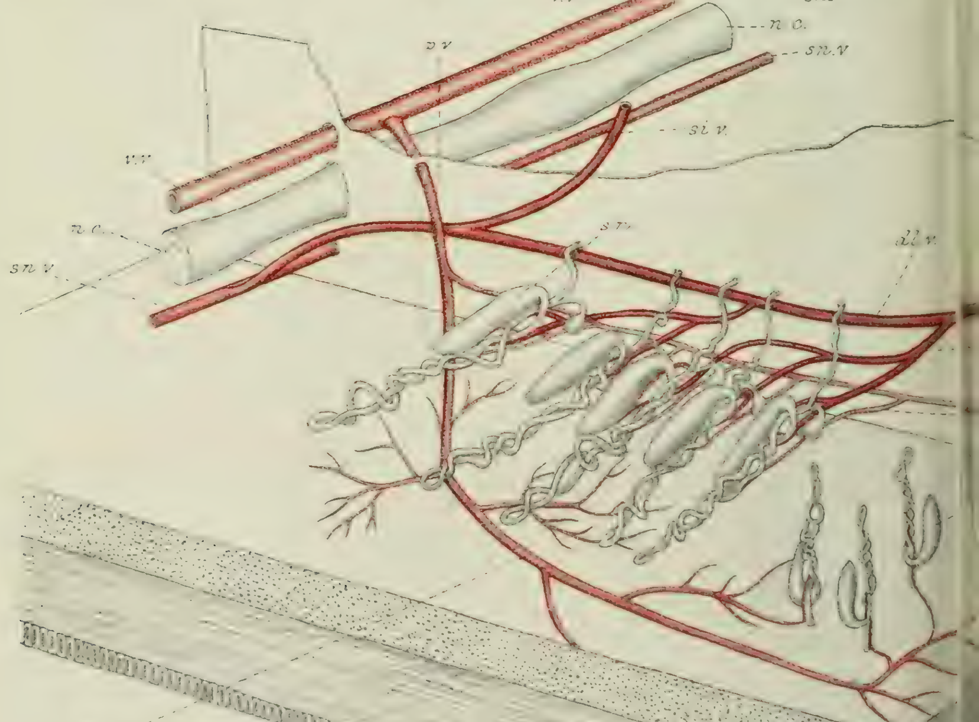
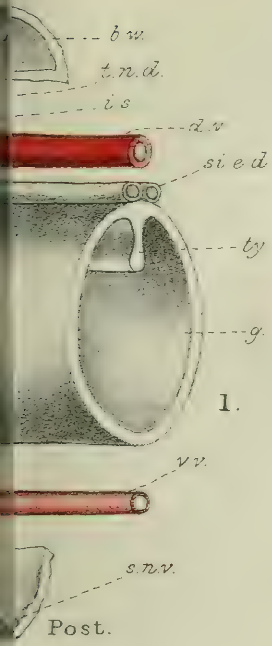




K.N. BAHL—NEPHRIDIA



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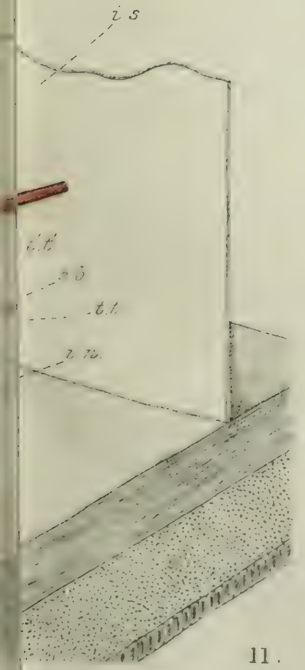

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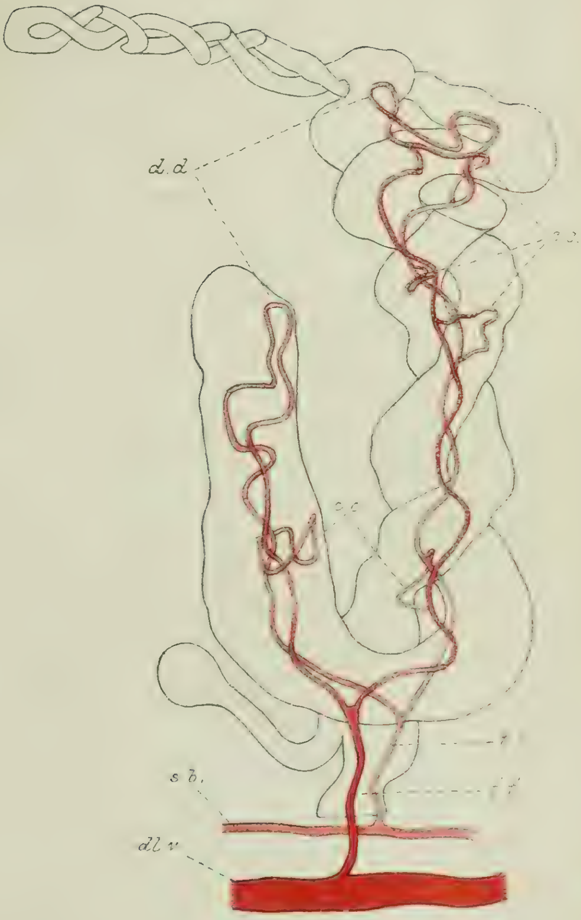

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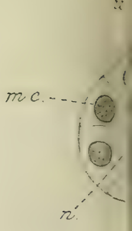
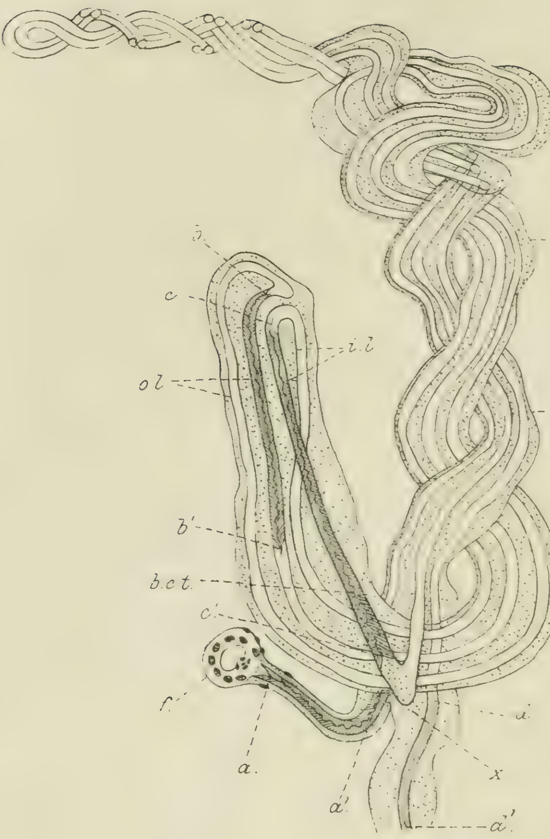
NDIAN EARTHWORM.





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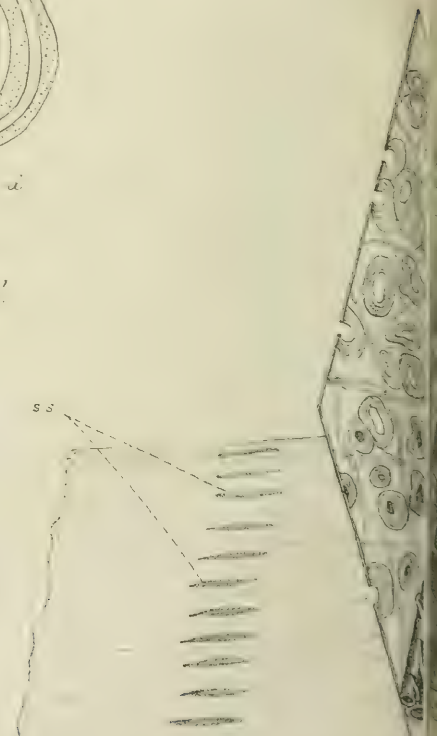

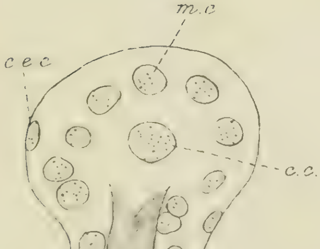

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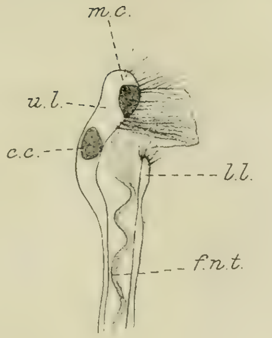

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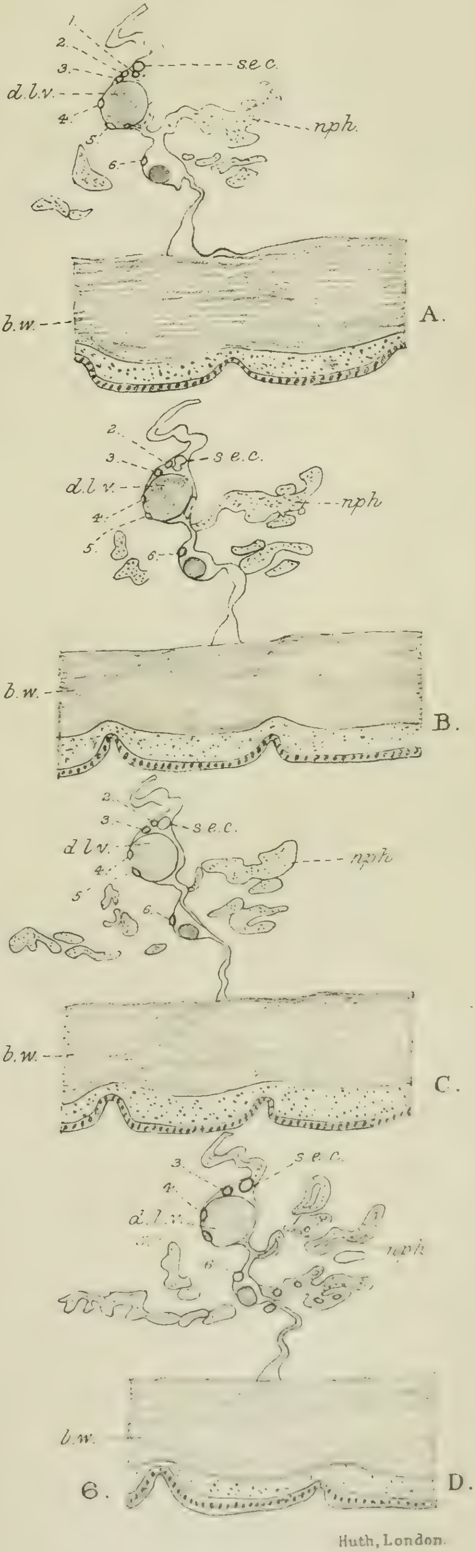

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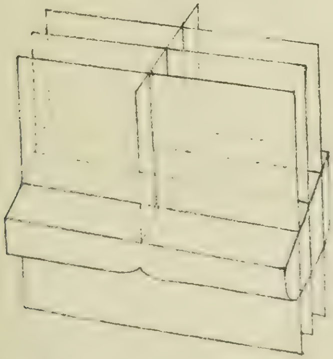

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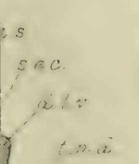

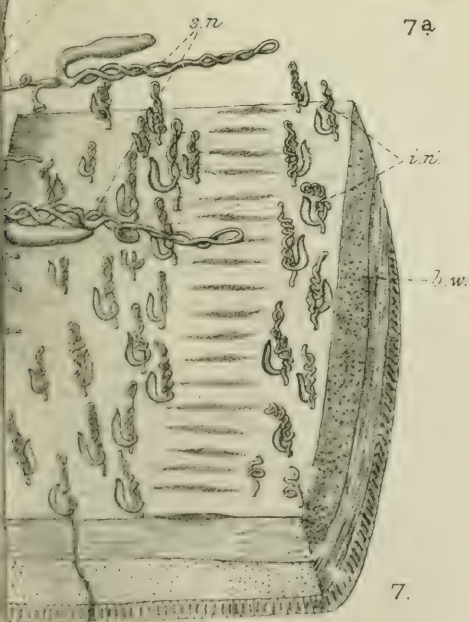

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DIAN EARTHWORM.

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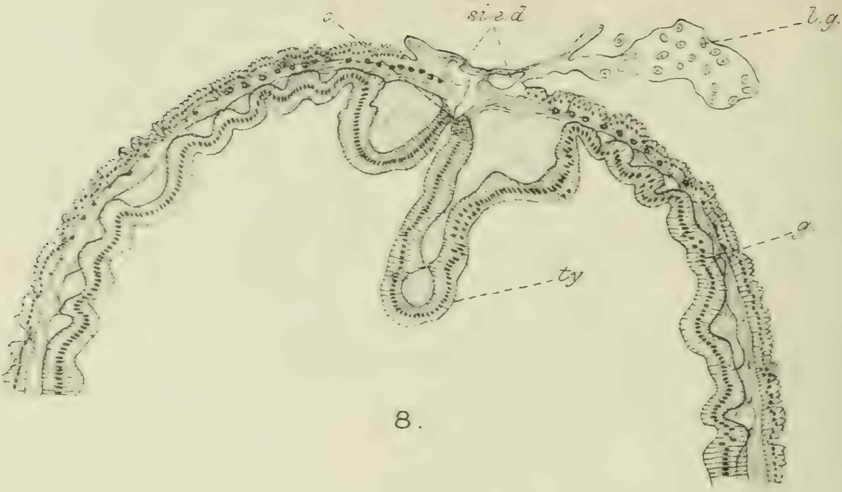

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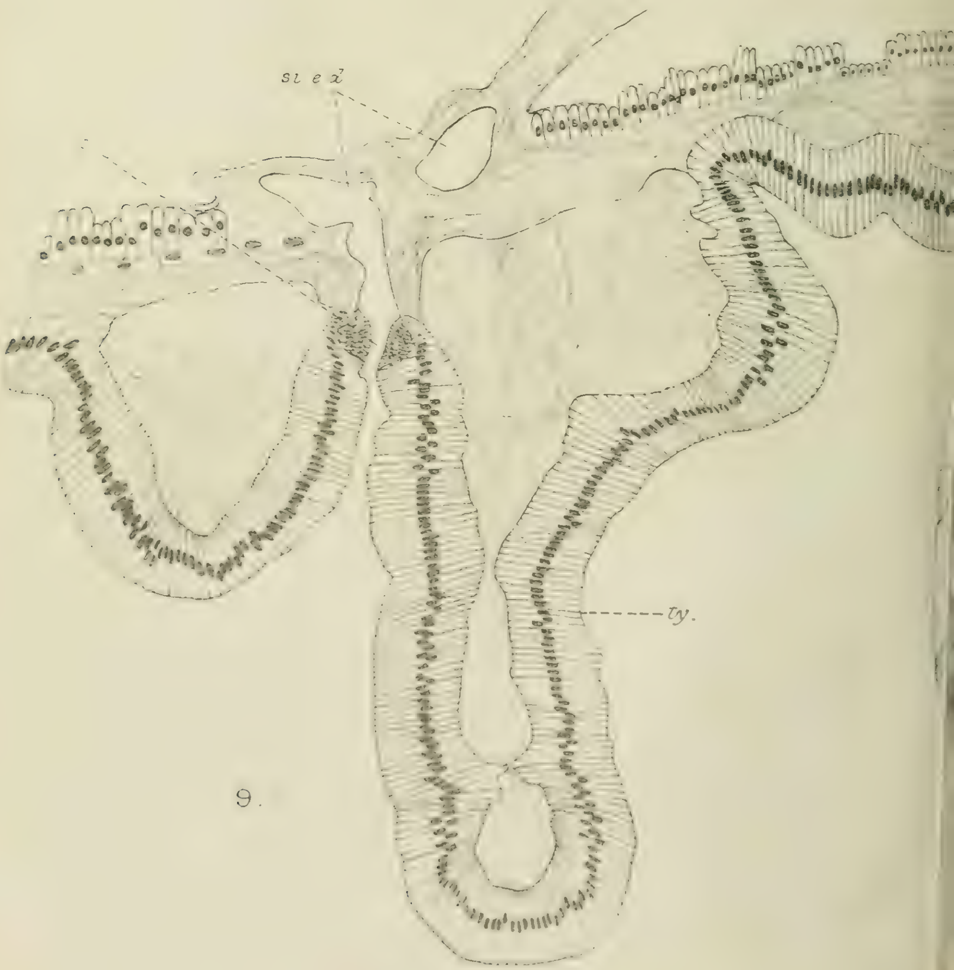

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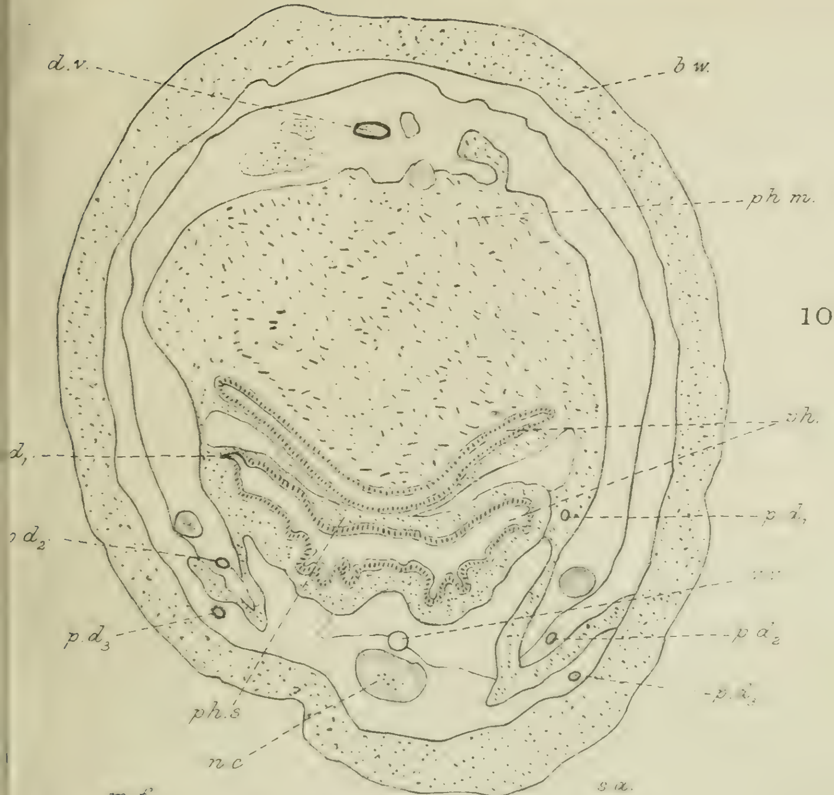

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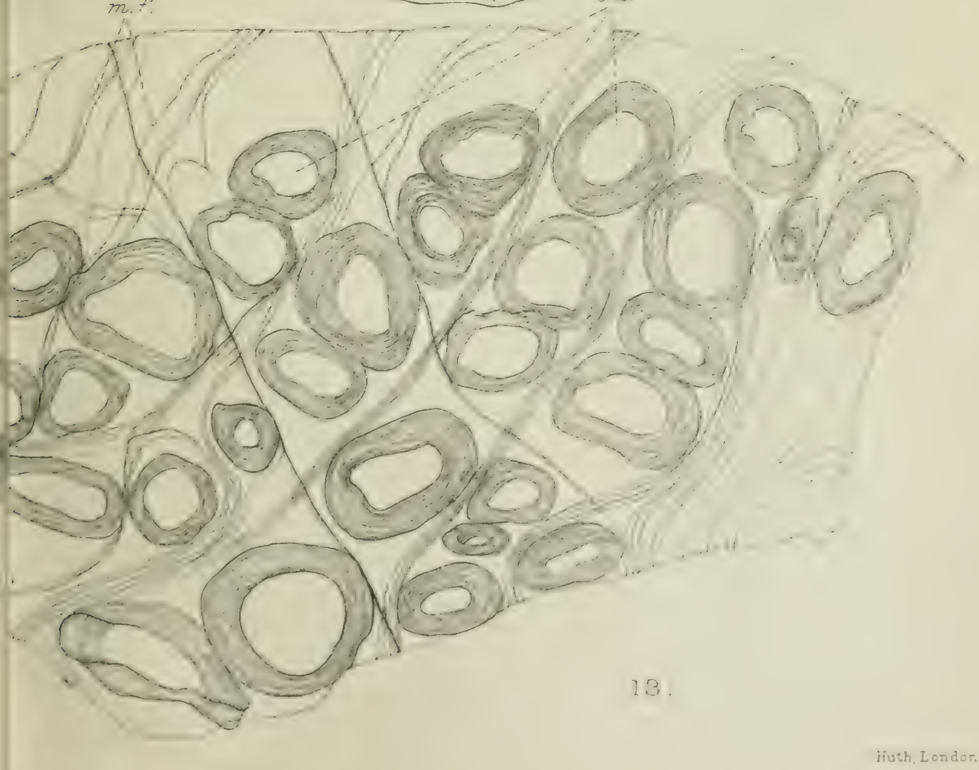

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NDIAN EARTHWORM.

Review.

Text-Book of Embryology: Vol. II, Vertebrata, with the exception of Mammalia. By J. Graham Kerr, Regius Professor of Zoology in the University of Glasgow. (Macmillan & Co., Ltd., London, 1919.)

The interesting Fish and Amphibians collected abroad by Prof. J. Graham Kerr and his friend Mr. Budgett have provided the material for a series of important contributions to our knowledge of the development of vertebrates in the pages of this Journal and elsewhere. Zoologists have therefore eagerly looked forward to the appearance of this the second volume of the 'Text-Book of Embryology,' of which the first volume—on the Invertebrata—was written by Prof. MacBride and reviewed four years ago; and they will not be disappointed, for Prof. Kerr has produced a strikingly original work of real merit. Unlike many text-books it is not a mere conscientious compilation, nor has the author aimed at writing a complete and comprehensive work of reference. Rather has he endeavoured to illustrate the fundamental results of the study of embryology, omitting mere descriptive detail, and avoiding the accumulation of data without special significance. As he truly remarks in the preface, such masses of detail "have tended to obscure general principles and incidentally to smother interest in one of the most fascinating of sciences." It must not be thought, however, that this handsome volume devoted to the Lower Vertebrata (Vertebrata, with the exception of the Mammalia) is filled with vague speculations; on the contrary, it conveys a vast amount of information, facts and relevant detail being set forth with admirable clearness to support the author's views.

The first chapter deals with early development from the zygote to the establishment of the germ-layers, and is followed by accounts of the skin and its derivatives (including the nervous system), the alimentary canal, the cœlomic organs, the skeleton, the vascular system and the external features of the body. There is then a chapter on adaptations to external environmental conditions, including fœtal membranes, and another on general considerations. Chapter X is devoted to a practical study of the embryology of the chick, while Chapter XI contains valuable "hints regarding the practical study of the embryology of various types of the lower vertebrates." The book closes with a useful appendix on methods. It is to be regretted that no introductory historical sketch is given of the growth of the science, pointing out the main steps in our knowledge and interpretation of the embryology of the Vertebrates. Such a review would stimulate the imagination of the reader, and help him to preserve a due sense of proportion in dealing with the many aspects of this vast subject.

Every chapter is interesting. The author, deeply impressed with the importance of his subject, boldly attacks controversial questions, defending his own theories with great skill. Thus, if the reader is not always persuaded, his interest is constantly kept alive, he is stimulated to further inquiry, and not seldom roused to opposition. Throughout, the work of the author and his colleagues on Lepidosiren and Polypterus is made to bulk very large, and if there is a weak point in the general treatment it is that the generalisations seem to be often based on too narrow a foundation. Valuable and instructive as these archaic fish may be for the science of embryology, it is difficult to avoid the conclusion that the too intensive study of these specialised types has sometimes warped the perspective of the author's outlook, biassed his deductions, and occasionally led him to neglect the work of others on types often much more suitable for the solution of the questions at issue. Thus, while we have nothing but praise for the masterly way in which he has marshalled his facts and dealt with some of the main features of the embryology of the Vertebrates, we are unable, unfortunately, to accept many of his general conclusions.

Discussing the origin of the mesoderm in Chapter 1, Prof. Kerr suggests that the place of origin of the enterocœlic pouches of Amphioxus, where transient stalks are formed uniting them to the endoderm, is represented in the higher forms, not by the more dorsal junction of mesoderm and endoderm on either side of the notochord, but by the more ventral or peripheral edge of the mesodermal plate (as shown in the accompanying diagrams, Text-figs. 1 and 2). Now it does not seem to have been convincingly shown that, in any Vertebrate, mesoderm is really derived from the endoderm at this peripheral margin, except in so far as the margin represents the stretched and modified lip of the blastospore. The derivation of the mesoderm in Vertebrates has been so much modified in the trunk by the accumulation of yolk and the specialisation of the blastoporal lip for growth in length, that it may be doubted whether its development will ever be fully understood until some painstaking embryologist studies the

TEXT-FIG. 1.

(This is Fig. 33 of the Text-book.)

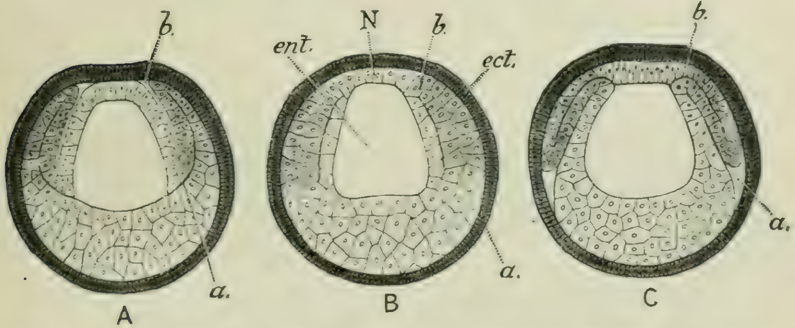
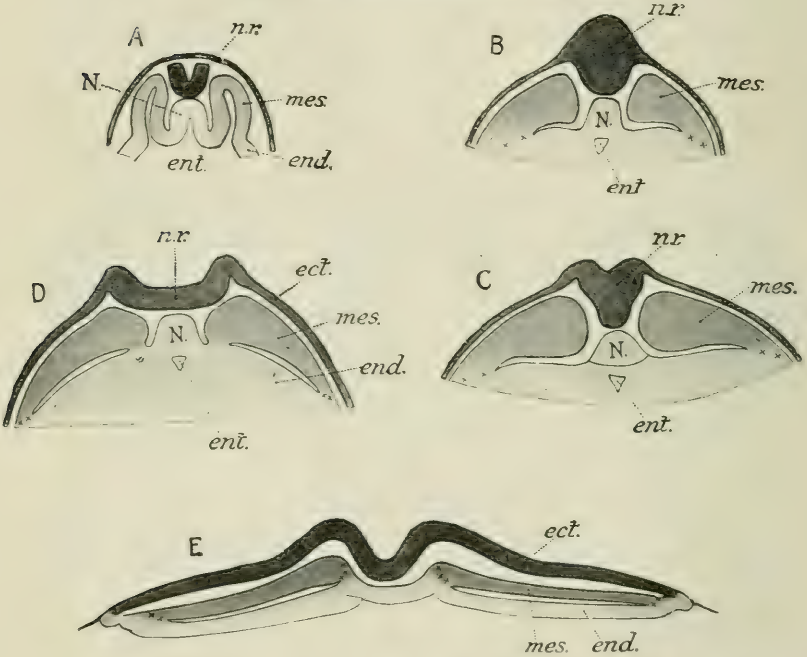


Diagram illustrating (*B*) the origin of mesoderm from endoderm in an Amphibian, and (*A* and *C*) the two methods of correlating it with the mode of mesoderm formation in Amphioxus. *a*, ventral and *b*, dorsal connection of mesoderm with endoderm. *N*. Neural tract. *ent*. Cavity of the enteron. *ect*. Ectodermal cell-layer.

whole question anew with the help of wax reconstructions. But surely the author's suggestion is quite inconsistent with what we know of the development of the mesodermal segments

TEXT-FIG. 2.
(This is Fig. 40 of the Text-book.)



Semi-diagrammatic transverse sections through the embryos of various vertebrates to illustrate the origin of the mesoderm. *A*, Amphioxus; *B*, Petromyzon; *C*, Lepidosiren; *D*, Amphibian; *E*, Elasmobranch.

in the anterior head region of the Elasmobranch, and still more with what we know of that of the Cyclostome, where they grow out as pouches on either side of the notochord just as in Amphioxus.

The development of the peripheral nervous system certainly deserves more detailed treatment than is here accorded to it;

on the other hand an excellent account is given of the three main theories of the origin of nerve-fibres and the evidence on which they are founded. The cell-chain theory supported by Balfour having been rejected, there remain 'the outgrowth theory' of His and 'the primitive continuity theory' of Hensen. The question is: Does a nerve-fibre grow freely outwards from its neuroblast to its destination—has it a growing point? Or is it gradually differentiated along a pre-existing protoplasmic strand? Prof. Kerr holds strongly to a modification of Hensen's view, believing that protoplasmic tracks first become established, then conduct nerve impulses, and that finally nerve-fibres become differentiated along them. He illustrates his theory with a striking simile: "As each successive passer-by causes a jungle pathway to become more clearly defined, so each passing impulse makes the way easier for its successors." Yet in spite of his ingenious arguments it is difficult not to believe that the evidence is on the whole more in favour of the theory of His. How else are we to account, for instance, for the "cone of growth" figured by Cajal at the tip of a developing fibre? Or, again, for the free endings—described by Neal—growing out of the nerve-cord in Elasmobranchs, and so like those seen in Harrison's well-known experiments on nerve-fibres growing in lymph?

It is interesting to read (p. 202) that in Lepidosiren not only the inner wall of the myotome produces muscle, but also the outer wall, for this occurs in Amphioxus as well; the doctrine of the cutis-layer must be revised. Probably because Lepidosiren is of little use for working out the segmentation of the head, the treatment of this important question is somewhat unsatisfactory. Following Fürbringer and other German writers it is assumed that one or several myotomes have been suppressed below the auditory capsule, that they do not correspond to the gill-slits, and that many myotomes have been pushed forward and reduced in the occipital region. Since this subject has been quite recently discussed in some detail in this Journal (vol. 63, p. 1), it is unnecessary here to repeat the evidence against these very doubtful propositions, and it need only be added that it is difficult to see any evidence whatever for the suggestion, made by Prof. Kerr, that the simplicity and uniformity of segmentation in the head region of the Cyclostomes and Amphioxus is secondary.

One of the best chapters is that dealing with the vascular system, especially the heart; and the account of the development of the heart of the bird, illustrated after the original drawings of Greil, strikes us as particularly good. Less satisfying is the part on the skeleton[1]—no doubt a very difficult subject to treat in a book of this kind. Yet something more might have been done to bring out "principles"—for instance, the distinction between the axial and the visceral skeleton. But it is probably with regard to the origin of the paired limbs that morphologists will find themselves most at variance with the author. Prof. Kerr would have us believe that the girdle is derived from a gill-arch and the limb from an external gill—a truly startling theory! To begin with, these external gills, found only in Polypterus, some Dipnoi and Amphibia, are obviously larval organs, and it remains to be proved that they are anything but precocious and specialised parts of the ordinary gill-system. There is no reason to suppose that they have ever occurred in Cephalochordates, Cyclostomes, or Elasmobranchs. They would appear to have arisen long after the median fins and probably long after the paired fins as well. Moreover, he would have us believe that from such a filamentous limb, like that of a larval Lepidosiren, arose the extended fins of fishes on the one hand and the pentadactyle limb on the other! For Gegenbaur's original "gill-septum theory" something may be said; it played, perhaps, a useful part in stimulating controversy and research. As knowledge has advanced, however, it has

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[1] With regard to Prof. Kerr's statement on p. 336, that there is no good reason why the shiny outer layer of ganoid scales should not be called enamel, it may be answered that ganoine appears to differ from true enamel in development, in minute structure, and in phylogenetic origin. That ganoine is merely modified bone can scarcely be doubted.

secured less and less support, and it is now generally admitted that the evidence of comparative anatomy and embryology is overwhelmingly against it (see R. C. Osburn, 'An. New York Ac. of Sci.,' 1906, and Goodrich in this Journal, vol. 50, 1906). Not all the special pleading indulged in by Prof. Kerr can shake this conviction. The marvellous correspondence in almost every detail of adult structure and embryonic development between the paired and the unpaired fins, the fact that they both arise as longitudinal folds, derive their muscles from the myotomes supplied by nerves from the ventral root system, and their blood from the somatic intersegmental vessels, is sufficient to show that the paired fin and the girdle which is developed in direct connection with its skeleton are fundamentally somatic or body-wall structures. The gill-arch, on the contrary, belongs to the visceral system, is developed from the splanchnic mesoderm, and derives its intrinsic musculature from the splanchnic lateral-plate-mesoderm supplied by the dorsal-root-system of nerves and its blood-supply from the arterial arches. Similarly the gill-septa and the gills developed in connection with them derive their muscles, nerves and blood-vessels from the same sources. Surely to seek to minimise the significance of these facts is to obscure those very "general principles" of vertebrate embryology which the author had set out to explain.

There is an excellent account of the development of the renal organs in Chapter IV, though it is disappointing not to find a description of the origin of the Müllerian duct in Dipnoi, which might be expected to throw valuable light on the still very obscure question of its homology. The author ably supports the "archinephric theory" originally put forth by Lankester (1877) and further developed by Sedgwick (1881), according to which "pronephros, mesonephros and metanephros are simply successive portions of a single elongated ancestral excretory organ possessing a duct and segmentally arranged serially homologous tubules." The introduction of the new term "opisthonephros" for the whole set of kidney tubules behind the pronephros in lower Craniates, to distinguish it from the differentiated mesonephros and metanephros of the Amniota, should be welcomed by embryologists and help to dispel a confusion in nomenclature.

When discussing the origin of the renal organs of the Vertebrata, Prof. Kerr reviews the whole question of the homology of excretory organs and genital ducts in the Cœlomata, and rejects the interpretation put forward by the reviewer in this Journal in 1894 and developed in several papers since. Briefly his view seems to be as follows: In an imaginary ancestral form somewhat resembling an Actinian in build, excretory tubes with flame-cells were present to remove waste products. When the enterocœlic pouches, whose walls are supposed to have given rise to the gonads, became shut off from the gut, the genital products, seeking out a weak point, escaped through the nephridia, whose inner ends happily came into proximity with the wall of the cœlom. Such points of temporary rupture gave rise to permanent funnels. Gradually excretory products came to pass through the funnels and the flame-cells disappeared. The paths of the genital cells and the excreta would then tend to become separated. Here the author's usual lucidity seems to desert him; for it is not at all clear from the text how these conclusions are to be applied to the Craniate Vertebrates. Judging, however, from the diagrams on p. 239, and here reproduced (Text-fig. 3), he would consider the renal tubule of a vertebrate to be formed by the grafting of a cœlomic funnel on to a nephridial tube—to be, in fact, a nephromixium! We need hardly point out that for this interpretation he gives not the slightest evidence. It has taken generations of embryologists and piles of literature to explain the real development of the vertebrate kidney, and if there is one fact which may be considered as firmly established, it is that the whole tubule as well as its longitudinal duct is directly or indirectly derived from the cœlomic wall. Thus Prof. Kerr persists in confusing two sets of organs of different origin and history under the one name "nephridium"; moreover, he appears not to have realised that there are two kinds of funnels opening into the cœlom—the nephrostome and the cœlomostome. The fact is that when discussing this question too much attention is generally focussed on the nephridium. There is really no

Text-fig. 3.
(This is Fig. 131 of the Text-book.)

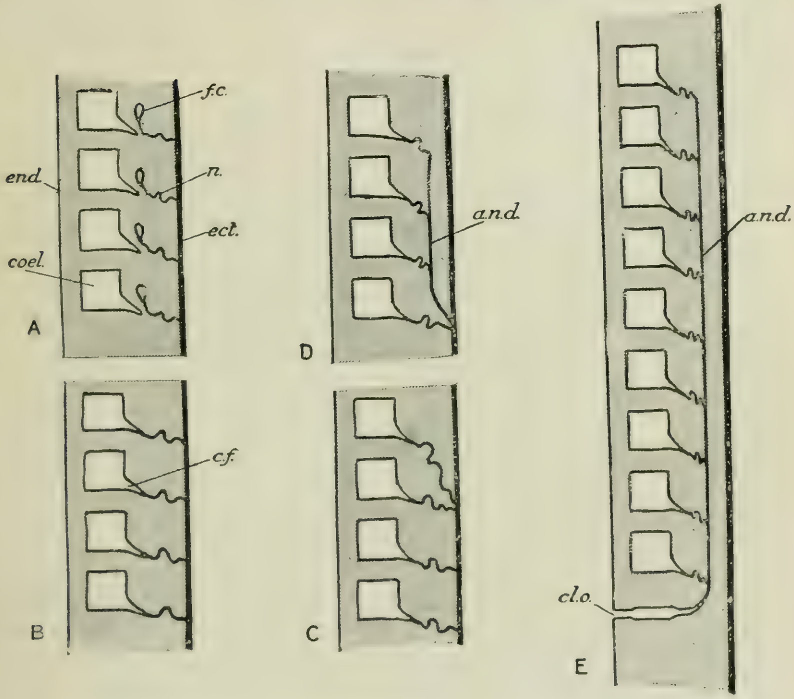

Diagram illustrating a possible mode of evolution of the archinephric duct. *a.n.d.*, archinephric duct; *c.f.*, cœlomic funnel; *cœl.*, cœlomic cavity; *cl.o.*, cloacal opening; *ect.*, ectoderm; *end.*, endoderm; *fc.*, flame-cell; *n.*, nephridial tube.

reason to mention the nephridium at all, nor need we call in hypothetical ancestors of dubious structure. It need only be granted that the remote ancestor common to the Vertebrata and other Cœlomata (Triploblastica) was provided with gonadial sacs, from whose walls were developed ducts for the escape of the genital products. These ducts are found in all the lowest grades of the Cœlomata, such as the Platyhelmia and Rotifera; in the Mollusca and Echinodermata, where the cœlom becomes extensive; and in the Nemertina, where the gonadial pouches and their ducts begin to become metamerically repeated. In the segmented worms, Oligochætes and Hirudinea, the ducts occur in all the fertile segments, and again persist in varying number and situation in the different groups of Arthropods. Thus, while these ducts are found in all the triploblastic Invertebrata, it is only in the one group Polychæta that a connection arises between them and the nephridium; and even among these worms the Capitellidæ and Nereidæ preserve quite independent cœlomic ducts, which, in the former family at least, open to the exterior to carry out the genital products in the way characteristic of the vast majority of the Cœlomata. Turning now to the Vertebrata, we find that the Craniates are provided with a similar series of tubules developed as outgrowths of the cœlomic wall, many of them (at all events in male Gnathostomes) retaining as genital ducts their original function. Is there any sound reason for refusing to accept the natural conclusion that these ducts are homologous throughout the Cœlomata? We certainly can find none in this book. That certain regions of the cœlom have become sterile and adapted to excretion, and that there has been a corresponding division of labour among the cœlomic ducts, some keeping to their primitive function and others becoming converted into renal tubules, among Arthropods, Molluscs and Vertebrates, is only another instance of that modification in the course of evolution so familiar with other organs, especially in a metameric series. In such forms the true nephridia naturally tend to disappear.

Much interesting and novel matter is given in the chapter devoted to developmental adaptations; while in that on general considerations there are good discussions on cellular continuity and the influence of yolk in development. But few embryologists, we think, will follow Prof. Kerr in his adherence to the "protostoma hypothesis" of Sedgwick. It is closely dependent on the theory that the Cœlomata are descended from an Actinozoon-like ancestor, whose mouth, surrounded by a nerve-ring and derived from the persistent blastopore, became elongated and closed in the middle, leaving openings at each end representing the mouth and anus. Further, the gastral chambers of the Actinozoon are supposed to give rise to the cœlomic pouches of the higher forms. It is a little disconcerting to find at the outset that gastrulation is not the commonest method of development among the Actinozoa, that the mode of formation of the ingrowing mesenteries bears no resemblance to that of the intersegmental septa in Cœlomates, and that their order of appearance is utterly unlike that of true segments, which develop regularly from behind forwards. Moreover, neither segments nor anus have yet appeared in the lowest Cœlomates. Surely the natural conclusion of an unprejudiced observer, who found that in the various groups a blastopore may not occur at all, or when it does that it may coincide sometimes with the mouth, sometimes with the anus and at other times with neither, that it is sometimes more dorsal and at other times more ventral, would be that this blastopore is, so to speak, merely a developmental device for securing the ready overgrowth of the endoderm cells by the ectoderm, and cannot have the phylogenetic significance so often attributed to it. However this may be, let us examine more closely the protostoma-theory as applied to the vertebrates. We must suppose that in some unexplained way and by a remarkable acrobatic feat the ancestor pushed one corner of its mouth below its head and the other below its tail. According to the theory the neural folds or edges of the neural plate would represent the lip of the elongated blastopore, and both the mouth and the anus should be formed within the neural area. Now it is doubtful whether the proctodæum ever arises within it, and the stomodæum certainly never does. The blastopore gives rise to the neurenteric canal, always enclosed by the neural folds behind. The adoption of the protostoma theory would also commit one to the "concrescence theory," according to which the whole dorsal region of the vertebrate body is supposed to have been formed by the fusion of the lips of the blastopore from mouth to anus along a line running through the floor of the neural canal. Space will not permit us to repeat the many serious—not to say fatal—objections brought forward against this view by competent observers. The whole question has been very efficiently dealt with by Assheton (see "Growth in Length" and other papers in this Journal and elsewhere), whose work is not even mentioned by Prof. Kerr in this connection. Assheton's contention that throughout the Vertebrates growth in length of the dorsal region takes place in front of the blastopore by proliferation from its dorsal lip, which is thus always carried backwards at the extremity of the tail, is, we believe, being generally accepted by embryologists. The true relation of the anus to the blastopore in Vertebrates is still very obscure.

If the reviewer seems to have dwelt at inordinate length on points of disagreement it is with no wish to find fault, but with a genuine desire to get at the truth by frank discussion. It is just on these questions about which there is difference of opinion that more research is needed, and we may hope that when Prof. Kerr is preparing a second edition of this valuable book he will revise some of his conclusions and give new and more convincing evidence for his views.

Finally we must express our admiration of the numerous illustrations adorning this volume, due for the most part to the skill of Mr. Kirkpatrick Maxwell. A large number of the figures are new to text-books and taken from the author's own works or those of his pupils. Author, illustrator and publisher are to be congratulated on having produced such an attractive volume.

E. S. Goodrich.

The Cytoplasmic Inclusions of the Germ-Cells.

PART VI. ON THE ORIGIN AND PROBABLE CONSTITUTION OF THE GERM-CELL DETERMINANT OF APANTELES GLOMERATUS, WITH A NOTE ON THE SECONDARY NUCLEI.

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With Plate 9 and 10 Text-figures.

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THE posterior pole of the ovum of many Arthropoda, and especially of Holometabolous Hexapoda, has been shown to contain a cytoplasmic aggregation which ultimately becomes segregated into the germ-cells during early segmentation stages (**4, 15, 21**). On account of this the polar aggregation has been called the germ-cell determinant. In this paper I have made some attempt to ascertain the origin and composition of the germ-cell determinant, and I have also taken the opportunity to draw attention to the remarkable accessory or secondary nuclei.

1. Material and Methods.

The biology of Apanteles and Microgaster has been treated in two other papers (**8, 9**).

Pupæ or newly emerged imagos were found to contain developing eggs in stages old enough for the purpose of this research. The abdomens of the insects were snipped off, teased open and fixed in a variety of fluids; Flemming-without-acetic acid (F.W.A.), Champy, Altmann, Benda, Kopsch (**7**), Golgi, Cajal, Mann, picro-nitric, Carnoy, Gilson-Petrunkewitsch were among the methods tried. Glycogen techniques were also used. The sections were stained in a variety of methods.

2. General Note on the Insect Ovary.

In insects the ovary consists of a number of tubes or ovarioles containing eggs in different stages of development, those nearest the oviduct or the posterior end of the ovariole being ripe, while those at the upper end or apex of the tube are minute. These stages are arranged in a linear series, and between two oöcytes one usually finds a group of nurse-cells (Text-fig. 4). The nurse-cells in most insects appear to be true germ-cells and are often connected to the growing egg by means of cytoplasmic rings, which show that the nurse-cells and the egg they nourish belong to the same cell-generation (**14**). In the ovariole the polarity of the egg is definite; the anterior pole of the egg lies towards the head of the insect, the posterior pole towards the genital opening. The germ-cell determinant is found to appear in the posterior pole of the egg, while the oöcyte nucleus lies towards the anterior pole of the egg-cell (Text-figs. 1 and 4).

At the apex of the ovariole all the cells are at first similar in appearance and size; as one follows the series down the tube certain cells are found to enlarge, apparently at the expense of the others, and become oöcytes, while the remaining cells either become nurse-cells, or form an epithelium around the oöcyte and are then follicle-cells (Text-fig. 5). According as to whether a germ-cell becomes a follicle, nurse- or egg-cell, the nuclear and cytoplasmic elements become characteristically altered as shall be shown below.

3. Previous Work.

Hegner (**14**) appears to be the only other observer who has examined the oögenesis of Apanteles glomeratus. In his valuable paper Hegner describes how at an early stage the oöcytes acquire an epithelium and are accompanied by a group of nurse-cells. In the nucleus of the young oöcytes the chromatin mass is very large, but later expands to form a spireme, and the nucleus becomes filled with chromatin threads—a condition which persists much later. When the oocytes reach their definitive size the chromatin threads contract into chromosomes, which apparently unite in pairs, as in Copidosoma (also described by Hegner), and become arranged side by side on an asterless spindle. Finally, the chromosomes condense to form a solid lump, as has been shown to occur in many parasitic Hymenoptera (**4**). Some time before the oöcyte nucleus breaks down to form the chromosomes, there appears within the cytoplasm of the anterior half of the oöcyte a great number of spherical bodies which are arranged in and below the zone of the large oöcyte nucleus. These are secondary nuclei, which were first described by Blochmann (**1**) in certain other Hymenoptera, and the substance within them stains like chromatin. The secondary nuclei are present for only a short time, having all disappeared before the nucleus condenses (see **4**, **15**).

In the Diptera, Lepidoptera, Hemiptera and in some Coleoptera, Stuhlmann (**22**) also describes such secondary nuclei.

Blochmann (**1**) considered that the secondary nuclei (he calls them "Nebenkerne") are formed by a process of budding from the oöcyte nucleus. Korschelt (**17**) considered they arose from epithelial cells. Loyez (**18**) thought that the secondary nuclei were not derived from the germinal vesicle by budding, but resulted from a fluid or granulose emission from the oöcyte nucleus. Hegner (**14**) appears to espouse this view, and concludes that "the oöcyte nucleus gives off materials into the cytoplasm which become enclosed by a membrane and develop into nuclear-like bodies." It should also be mentioned that Loyez considers that the secondary

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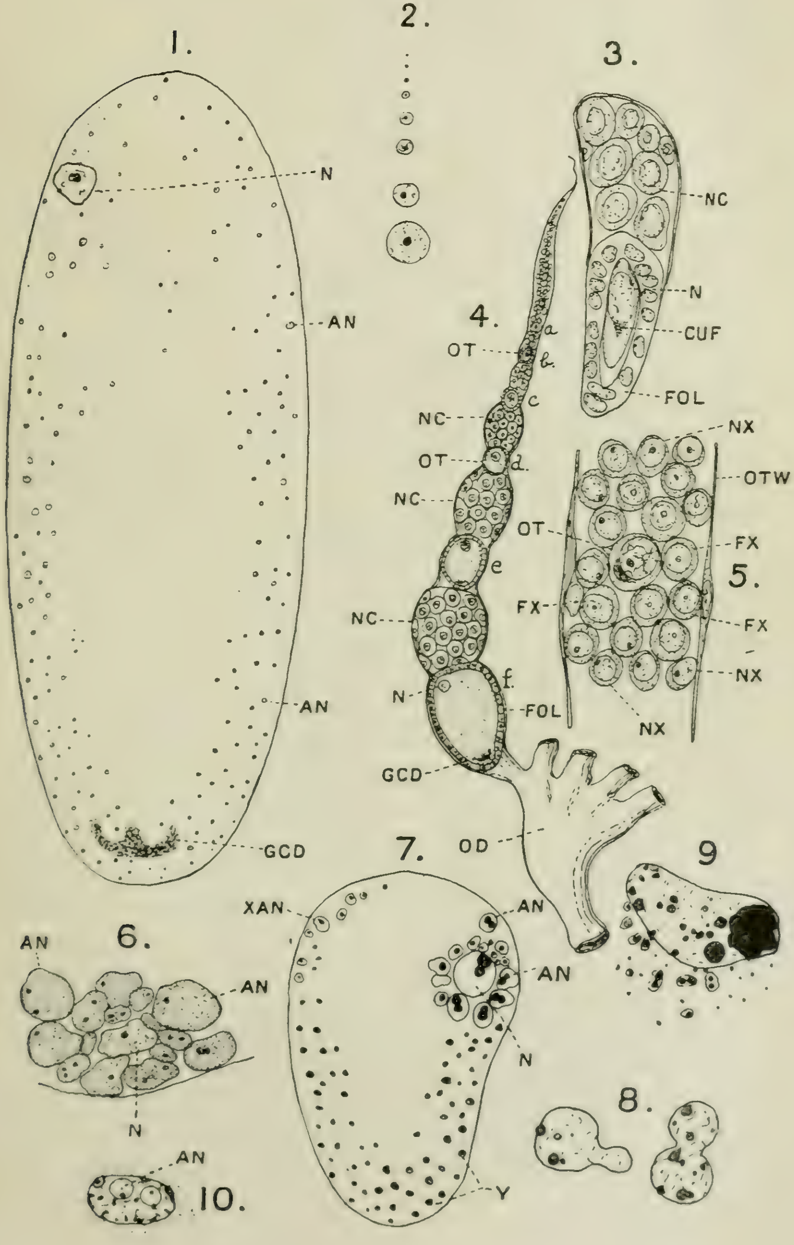
DESCRIPTION OF TEXT-FIGS. 1–10.

Fig. 1.—Diagram of egg of Apanteles glomeratus showing *N.*, the head nucleus; *AN.*, the accessory or secondary nuclei at the periphery of the egg; and *GCD.*, the germ-cell determinant. Fig. 2.—Showing the origin of a secondary nucleus of Apanteles from a solid chromatoid granule. The linin and nuclear membrane develop around the latter. Fig. 3.—Oöcyte and nurse-cells (*NC.*) of Copidosoma Buyssoni (Mayr.), after Silvestri (**21**). At *CUF.* is Silvestri's cloud of granules, supposed to give rise to the germ-cell determinant. *N.* Nucleus; *FOL.* Follicle cells. Fig. 4.—One egg-tube or ovariole of Apanteles glomeratus, showing linear arrangement of developing oöcytes (*OT.*), and groups of nurse cells (*NC.*). At *N.* is the nucleus of the oldest oöcyte, and at *GCD.* the developing germ-cell determinant. *OD.* Oviduct. Fig. 5.—Diagrammatic figure of upper part of ovariole to show origin of oöcyte nurse-cells and follicle-cells. At *OT.* is one inner cell enlarging to form an oöcyte; the cells marked *FX.* will eventually form the cellular layer of the egg (or follicle), while those in the region *NX.* will become nurse-cells. *O.T.W.* Ovarian tube wall. Fig. 6.—Part of oöcyte of Camponotus herculeanus to show primary or head nucleus (*N.*), and around it the secondary nuclei (*AN.*). Note different appearance of latter. After Hegner (**14**). Fig. 7.—Oöcyte of Myrmicina sp. showing the primary nucleus at *N.*, and around it a group of secondary nuclei (*AN.*) These stain like the head nucleus (*N.*) At *XAN.* is a group of secondary nuclei which have wandered from the region of the head nucleus. *Y.* Yolk. After Buchner (**3**). Fig. 8.—Two secondary nuclei of Rhyssa (Ichneumon) to show budding and amitosis. After Buchner (**3**). Fig. 9.—Nucleus of egg of Rhyssa, showing apparent origin of secondary nuclei, by expulsion of nucleolar material from the head nucleus. After Buchner (**3**). Fig. 10.—Nucleus of egg of Rhyssa, showing three accessory nuclei (*AN.*) inside. After Buchner (**3**).

TEXT-FIGS. 1–10.



nuclei may have some function in the formation of the yolk. All observers find that the secondary nuclei sooner or later disappear; exactly how has not been determined.

In other insects Hegner (**15**) has studied the cytoplasmic inclusions—mitochondria and other bodies; this, however, was not done in Apanteles, since his material was fixed in Carnoy and Bouin. In the beetle Leptinotarsa decemlineata, this observer describes the cytoplasm of the very young oöcytes as homogeneous, figuring no inclusions. After a short time the oöcytes form a linear series in the ovariole, and soon there appears in the cytoplasm a number of granules staining with the crystal violet after Benda's method. The cytoplasm growing, becomes filled with more and more granules which become situated near the periphery. The central cytoplasm is of a homogeneous nature, the peripheral crowded with granules and spherical bodies of various sizes. Hegner thinks that the mitochondria lying near the periphery increase in size, swell up, lose their affinity for crystal violet, and finally form the large yolk-granules.

With reference to the mode of origin of the germ-cell determinant in Leptinotarsa, Hegner thinks that the mitochondria might be responsible for its appearance. He could not determine the mode of origin of the germ-disc, but considered that it might be derived from—(1) the cytoplasm of the egg, (2) the cytoplasm of the nurse-cells, (3) the chromatin of the germinal vesicle, (4) chromatin from the nurse-cells, (5) nucleolar substance from the germinal vesicle or nurse-cells or both, and (6) bodies of a mitochondrial nature.

It may be safely remarked that with the exception of yolk and Golgi apparatus, Hegner mentions as the possible source of origin of the germ-cell determinant every likely cell constituent known to cytologists. Hegner apparently does not feel in a position to eliminate from his list any one of the possible sources of origin of the determinant but appears to be attracted by the last section—No. 6.

Silvestri (**21**), using sublimate-alcohol-acetic acid as fixative, describes in Copidosoma buyssoni, a polyembryonic

Chalcid, the origination of the germ-cell determinant as "una sostanza un pò' più colorita dell' ooplasma e meno della cromatina e formante come una sorta di cuffia alla parte posteriore della vesicola. Di questo stadio io ho pochi preparati e non molto belli, perciò non mi credo autorizzato ad affermare in modo assoluto la presenza di tale sorta di cuffia alla parte posteriore della vesicola." In Text-fig. 3 I have copied one of Silvestri's figures, showing at *CUF.* the "sorta di cuffia," which he thinks may have something to do with the origin of the germ-cell determinant. As will be shown below, Silvestri's juxta-nuclear "cuffia" is really unconnected with the germ-cell determinant, but is the partly collapsed remains of the mitochondria. Martin (**19**) also tries to find some condition inside or near the nucleus to account for the origination of the germ-cell determinant, figuring the germ-cell determinant as appearing on that side of the nucleus which contains a darker mass of chromatoid substance. Martin's evidence is not satisfactory. The chromatoid mass on one side of the nucleus is merely the typical bouquet stage figure of the maturation prophases, and there is no evidence that this is connected with the appearance of the germ-cell determinant.

Paul Buchner (**3**), in a recent exhaustive study of the accessory nuclei in the hymenopterous oöcyte, has examined Solenius vagus, Andrena sp., Bombus agrorum, Camponotus and Myrmecina sp., Rhyssa and Allantus sp., Arge pagana and Tenthredo mesomelas. In all these forms he finds secondary nuclei. His paper does not enter specially into the questions surrounding the germ-cell determinant. Buchner's conclusions with regard to the secondary nuclei are as follows: "Die akzessorischen Kerne enthalten keine Chromosomen, im übrigen gleichen sie dem Hauptkern, indem sie mit Liningerüst, Nukleolen, Enchylem und Membran versehen sind." Again, "Die akzessorischen Kerne sind auf anfangs nakt im Plasma liegende Chromatingranula zurückzuführen. Um diese entwickelt sich Enchylem Membran und Gerüst, während sie selbst zu den Nukleolen des akzessorischen Kernes werden." And, "Chromosomen sind auch zur Bildung eines Metazoenkernes nicht unumgänglich nötig, sondern lediglich Chromatin." In addition he considers that "Chromosomen und Nucleolen sind in der Eizelle in hohem Grade unabhängig voneinander."

The last statement is possibly thought to get over a difficulty which I have emphasised below in my discussion on the origin of this chromatin which gives rise to the secondary nuclei (page 148).

Buchner shows clearly that the secondary nuclei originate from grains of chromatin which in some cases are large enough to be found passing through the nuclear membrane. In Text-figs. 7, 8, 9 and 10 I have given four of Buchner's most remarkable figures, illustrating forms which differ from Apanteles, and which give more definite evidence as to the origination of the accessory nuclei.

In another recent paper (**20**), J. Nusbaum-Hilarowicz discusses the behaviour of the mitochondria during the oögenesis of Dytiscus marginalis. He has demonstrated a Golgi apparatus in the egg. In the oögonia there is a cytoplasmic aggregation near the nucleus, which he calls "Idiozomreste," and the mitochondria tend to lie around this region. During the oögonial divisions the mitochondria, which are fine and rod-like, become partly caught up in the astral rays. In Dytiscus the young eggs are connected to the nurse-cells by a zone of cytoplasm, as has been shown by Korschelt (**16**), and Nusbaum-Hilarowicz shows that most of the mitochondria lie in and partially form such cytoplasmic stalks. Besides yolk-granules the author describes the formation of large fat-granules.

The Golgi apparatus was demonstrated by a modification of Kopsch's method as follows:—Fix whole ovary (or ovary + viscera surrounding it) for two hours in this mixture: 1 part of 2 per cent. OsO_4, and 3 parts of corrosive sublimate saturated in normal saline. Then transfer, after slight washing in water, to a 2 per cent. solution of OsO_4. The tube containing the latter is kept ten days in a thermostat at a constant temperature of + 23° C., afterwards slightly washed in water, dehydrated, cleared, and embedded in wax. Sections may then be treated in turpentine to remove the bulk of the fat stain, if desired.

Except for the raising the temperature this method is similar to one recommended by me elsewhere (7). I find this method does not always succeed, but it does sometimes give results where ordinary Kopsch treatment fails.

4. Secondary Nuclei of Apanteles.

The secondary or accessory nuclei of the oöcyte of Apanteles arise after the egg has grown till it has lost its round shape and become oval and elongate (Text-fig. 4, *e* and *f*). In the most perfect preparations of the ovary that one can make these peculiar bodies are found to arise as minute solid chromatoid granules; they appear in the egg-cytoplasm far removed from the true nucleus. In Text-fig. 2 a number of stages showing the manner of origin of the accessory nucleus are given; at first the chromatic granule is solid and naked, but soon a distinct nuclear membrane appears around it, and the structure looks like a sphere surrounded by a thin ring. Later the extra-spherical ring (nuclear membrane) grows and gradually a linin network may be seen. Eventually the sphere of chromatic substance after growing may break up into several parts, or it may remain discrete. In Apanteles the larger secondary nuclei resemble the true or head nucleus to the smallest details, and one who did not know that these bodies had originated in a peculiar manner would unhesitatingly pronounce them to be normal nuclei, capable of undergoing mitosis (Pl. 9, fig. 11, *S.N.*).

Some time before the ovarian oöcyte has become ripe the secondary nuclei disappear by a process of degeneration or chromatolysis. The nuclear wall disintegrates and the other secondary nuclear elements become indistinguishable from the ground cytoplasm.

5. THE MITOCHONDRIA.

All three ovarian elements, egg, nurse- and follicle-cell, have a common derivation as explained. The indifferent cell is drawn in Pl. 9, fig. 1; the mitochondria at *M.* are fairly conspicuous granular formations. In each ovarian element the mitochondria have a special character; in the follicle-cells they remain unchanged (Pl. 9, figs. 6, 11); in the nurse-cells they change profoundly, becoming extremely numerous and fine. They are then closely grouped around the nucleus. In Pl. 9, fig. 7, is a young nurse-cell; its mitochondria are not much finer than those of the indifferent cell, but are more numerous. Gradually these mitochondria divide and multiply till they form a dust-like halo around the nucleus (Pl. 9, figs. 8 and 9, *M.*).

Inferior fixation causes the mitochondria to collapse and fuse partly to form a number of concentric rings around the nurse-cell nucleus; even in the best preparations the very fine mitochondria form a thick impenetrable layer around the nurse-cell nucleus. The rest of the nurse-cell cytoplasm has some mitochondria in it, but less closely packed.

In the follicle-cells the mitochondria keep large, and much like those of the indifferent cell (see Pl. 9, figs. 4, 5, and 11).

The history of the mitochondria in the egg is quite different. In the young oöcyte undergoing the early prophases of the maturation divisions as in Pl. 9, figs. 2 and 3, the mitochondria are coarsely granular and tend to lie around an archoplasmic sphere (*A.R.*). The Golgi apparatus will be found to lie upon this sphere, but so far I have been unable to demonstrate it either by Kopsch or Cajal technique. In the larger insect Dytiscus, a water-beetle, Nusbaum-Hilarowicz has shown a Golgi apparatus to be present in ovarian and follicle cells. It takes the form of small rings or half hoops very like that of Helix (**5**) or Lepidoptera (**6**). The granular oöcyte mitochondria gradually spread around the nuclear periphery as in Pl. 9, fig. 4. About this time they lose their granular appearance and become at first elongate and then filiform.

In Pl. 9, fig. 5, this is just happening. After this stage the granules tend to pass outwards through the cytoplasm, often leaving a space around the nucleus, *CS.* in Pl. 9, fig. 6. Notice in this figure the difference between follicular (*F.*) and oöcyte (*M.*) mitochondria. Just after the stage in Pl. 9, fig. 6, the mitochondria pass throughout the entire egg cytoplasm (fig. 10), and yolk begins to appear at the periphery of the oöcyte. In later stages the mitochondria increase enormously in importance and may form thick matted masses in the egg (Pl. 9, fig. 11, *M.*).

6. The Yolk (Deutoplasmogenesis).

After the mitochondria have spread throughout the egg and have become filamentary, there ensues a period in which the periphery of the egg becomes markedly well supplied with a matted dense cloud of mitochondria (Pl. 9, fig. 6).

As yet no yolk has appeared; the first sign of yolk is found in the appearance of tiny spheres close to the periphery of the egg, beneath the follicle wall and in the mat of mitochondria. As the egg grows older (Pl. 9, fig. 11, *Y.D.*) the yolk-spheres become much larger, but still are found only around the peripheral layer on the oöcyte.

Subsequently the spheres spread inwards, becoming larger as they do so, and eventually the entire substance of the egg is found to be loaded with the spheres. It is during this process that the secondary nuclei attain their largest size; as has been remarked, the latter lie on the periphery of the egg as shown in Pl. 9, figs. 11 and 12, and in Text-fig. 1, and the inference is that they are placed there in order that their influence may be used in causing the formation of food-yolk.

In no case did I find anything which would suggest that yolk was formed by metamorphosis of the egg mitochondria, and I consider that this is not a usual occurrence in oögenesis.

7. The Composition of the Germ-Cell Determinant.

The following fixing and staining tests were carried out on the germ-cell determinant.

Chrome-osmium.—Fixation of the ovary in Flemming-without-acetic and staining in iron-hæmatoxylin and orange G or in safranin gave the result drawn in Pl. 9, fig. 12. The determinant before staining looked a yellowish-brown colour, and after staining a dark brown to black colour. It was made up of granules, in the early stages at least (such as in fig. 12), and these grains were spherical and seemed something apart from the rest of the egg cytoplasm. No cytoplasmic reticulum to be seen in preparations fixed as above. The mitochondria appeared in the egg as fine filaments, generally several tangled and matted as in Pl. 9, fig. 11, *M.*

Mitochondria were found quite close to and often seemingly at the edge of the substance of the germ-cell determinant. Yolk-discs were yellowish by this method and beautifully preserved; they did not actually lie in the substance of the germ-cell determinant, but some passed around under the posterior end of the determinant as at *Y.D.X.* in Pl. 9, fig. 12.

Kopsch's Method.—This method consists in leaving material in 2 per cent. OsO_4 for two weeks. By it fat and generally Golgi apparatus are blackened; lecithin and ground protoplasm go a yellowish to light brown in Kopsch. In Pl. 9, fig. 13, I have drawn the posterior pole of an egg at the same stage as in Pl. 9, fig. 12.

This test shows that the area of activity of the formation of the germ-cell determinant is not confined to the inner region of the pole of the egg, as in Pl. 9, fig. 12, but is spread over the entire posterior pole; this is indicated by the fact that while the darkest part of the egg lies at *G.C.D.*, in Pl. 9, fig. 13, the area immediately below and at the sides is much darker than the cytoplasm not in the region of the germ-cell determinant. No structure or area in the egg at this stage could be made to go black with OsO_4, the natural inference consequently being that the germ-cell determinant does not contain fat (olein).

Fixation of the ovaries in picro-nitric has the effect of removing all the yolk and apparently all signs of mitochondria.

Even though this treatment removes all yolk-discs as shown in Pl. 9, fig. 14, it has not the effect of removing all the germ-cell determinant or of making it less obvious after staining. The natural inference from this is that while the germ-cell determinant may possibly contain some free lecithin, its main bulk consists of some organic substance other than lecithin or fat.

Fixation of the oöcytes in Carnoy or absolute alcohol and staining for glycogen by the iodine method and by Best's carmine did not succeed in tinging the region of the determinant in any way suggesting that it contained glycogen, while the fact that the determinant is well preserved by fixatives known to dissolve away glycogen leaves us with the conclusion that the determinant consists of something other than glycogen.

Methods such as that of Kopsch and Cajal for the Golgi apparatus failed to reveal this latter either in egg or in germ-cell determinant. That this shows that the oöcyte of Apanteles does not contain a Golgi apparatus is a conclusion which I am unwilling to draw, but from other tests, such as that of Carnoy's fluid (known to dissolve the Golgi apparatus of other eggs), I feel justified in concluding that the Golgi apparatus does not form a part of the germ-cell determinant (see also pp. 140 and 149).

Especially after fixation of the ovary in alcohol and acetic fixatives, the germ-cell determinant stains basophil, but this alone does not show that it contains chromatin. We have no evidence that the germ-cell determinant is even partly formed of chromatin, while we cannot observe any occurrences in the history of the formation of the determinant which lead us to associate its inception and growth with either head nucleus or secondary nuclei. It should be mentioned that it is not possible to obtain completely satisfactory evidence that the germ-cell determinant does not contain chromatin, but I feel sure that chromatin in bulk masses does not take part in forming the determinant. Granulose emissions of chromatin might pass into the determinant from the nucleus without the fact being observable. Since, however, the determinant does not stain quite like chromatin one can only believe that the evidence is against the assumption that this protoplasmic aggregation is formed partly or wholly of chromatinic substance.

8. Discussion.

(a) Germ-cell Determinant.

In this paper it has been shown that the germ-cell determinant in Apanteles glomeratus is probably formed of albuminous material as opposed to chromatin, or storage substances such as fat, yolk, or glycogen. The exact significance of this is difficult to determine, but it seems probable that germ-cell determinant is a form of metaplasm, and that its purpose is that of providing nutriment in a special form, enabling the germ-cells to remain independent of the other cells, such as those of the germ-layers, while the latter are undergoing their organogeny changes. Histo-chemically the germ-cell determinant consists of proteid, which, when coagulated by alcohol, acetic acid, corrosive sublimate, etc., becomes insoluble in either water, alcohol, or xylol and chloroform.

No direct connection can be shown to exist between the germ-cell determinant and the chromatin of the oöcyte nucleus or the follicular nuclei. It must be admitted, however, that this does not show positively that the germ-cell determinant contains no chromatin. The germ-cell determinant, while being basophil, never stains in quite the same sharp way as the chromatin of the nucleus when one uses dyes like methyl green or safranin; for this reason alone one is justified in considering that the germ-cell determinant is at least not formed of nucleo-proteid of the same constitution as that found in the nucleus.

(b) The Secondary Nuclei.

The secondary or accessory nuclei of the insects are very remarkable structures, and there is no doubt that they throw a great deal of light on the general structure of a nucleus. Among the several interesting facts which have been ascertained with regard to these accessory nuclei, none is more remarkable than that which shows them to be of nuclear origin and to contain chromatin. The small granules found in the cytoplasm which give rise to the accessory nuclei stain exactly like chromatin, and personally I have no doubt that such granules are chromatin. These granules lie naked in the cytoplasm, but later form around themselves a nuclear membrane and a linin network. The enchylema or nuclear sap just after formation is quite hyaline, and the linin appears later. Buchner has pointed out that the study of the accessory nuclei shows that the presence of chromosomes is not a sine quâ non for the building up of a nucleus. This statement, however, is not quite true, nor is it a new suggestion. What is a chromosome? It is merely a mass of chromatin, which appears in the nucleus during karyokinesis, and I believe that it is not possible to discriminate between a large piece of extruded chromatin (Text-fig. 9) and a chromosome in the sense meant by Buchner. We also were aware previous to Buchner's work that single chromosomes could form nuclei (**2**, **6**).

By far the most important result of work on the accessory nuclei is the fact that chromatin, apparently in large quantities, can be produced in and budded off from a germinal vesicle without affecting the subsequent results of development. We have distinctly satisfactory evidence that the chromatin forming the accessory nuclei passes out of the germinal vesicle, but none that any passes back, while there is good evidence that the accessory nuclei degenerate eventually.

The egg nucleus in many insects, of which Apanteles is an example, becomes partly decentralised; this is to say, the nucleus, instead of influencing various processes of oögenesis from afar, sends pieces of itself into the furthermost regions of the egg, which carry on part of the vegetative functions at least of the chromatin in the ordinary nucleus. The head nucleus is able to produce chromatin for this purpose, without endangering the complete exercise of its proper functions in later stages of the germ-cell cycle. The chromatin so produced must apparently be part of the chromosomes of the egg nucleus, so that one seems led to believe that the chromosomes are able to part with some of their substance without detriment. This phenomenon, as also the appearance of giant germ-nurse cells in Testacella (**5**), is in some way connected with the growth faculty of the substance " chromatin." In the case of Apanteles and of Testacella, the new chromatin so produced might by some be looked upon as trophochromatin, or specialised for purely trophic or vegetative functions.

In certain cases, such as that of Apanteles, the secondary nucleus exactly resembles the head nucleus except for size. This applies also in such an example as Rhyssa, in Text-fig. 7. In other cases, however, the secondary nuclei may not resemble the head nucleus, and may be much larger than the latter, as in Text-fig. 6, of Camponotus. This difference in appearance seems due to the difference in the arrangement and deneness of the linin network in the accessory nuclei; in the latter the karyolymph is more dense, or the linin is more closely woven.

It seems indicated that the secondary nuclei are formed from true chromatin of the head nucleus, and that their function is connected with the growth of yolk or other materials in the egg-cytoplasm. All staining tests appear to show that such chromatin extruded from the nucleus is exactly similar in its histo-chemistry to the chromosomes. It should be mentioned that Dendy (**3a**) especially shows that in several sponges solid lumps of chromatin are extruded from the nucleus of the egg during oögenesis, this also without affecting subsequent development of the organism.[1]

I mention these facts, not as a disbeliever in the useful " chromosome hypothesis," but because I consider that chromosome theorists might be interested enough to comment on such peculiar occurrences, which seem fairly wide-spread, and which must be explained by workers on chromosomes.

---

[1] I have seen these preparations recently.

(c) The Protoplasmic and Deutoplasmic Inclusions of Insect Germ-Cells.

In the Lepidoptera it has been shown that the male germ-cells contain both mitochondria and Golgi apparatus (**6**); I consider that the sickle-shaped bodies drawn by me in the figures of the sperm-cells of moths are homologues of the Golgi apparatus or "apparato interno reticolare" of the mammalian nerve or germ-cell. In my work on moths I failed to demonstrate such sickle-shaped bodies in the öocyte; likewise in this present paper all my efforts to show such bodies in the oögenesis of Apanteles have failed.[1] It is therefore interesting that a Polish worker, Nusbaum-Hilarowicz, should have succeeded in demonstrating a Golgi apparatus in the young oöcytes of another insect—the large water-beetle, Dytiscus. This has been done by using a special new modification of Kopsch's method. In examining male germ-cells of Apanteles I have found the Golgi apparatus, and I believe that some special modification of Kopsch's method should also show the apparatus in the egg, as has been done by me in Limnæa and other molluscs. This matter, however, does not affect the issues discussed in this present paper.

In Apanteles the oöcyte contains no fat-droplets such as have been shown to occur in the egg of Dytiscus, but in both insects yolk-spheres are present and appear in the same region of the egg cytoplasm. Nusbaum-Hilarowicz shows that yolk-spherules and fat-droplets differ in their histo-chemical reactions, as has already been pointed out elsewhere (**7**).

9. Conclusions.

Composition.

(1) The germ-cell determinant of Apanteles glomeratus is formed almost wholly of a basophil albuminous proteid somewhat like that of the ground protoplasm of the egg-cell, only much more dense and definitely basophil.

---

[1] I have made preparations of Stenobothrus ovary showing Golgi elements, by Cajal's method and by Mann-Hopsch. Apanteles is small and difficult to manipulate by Golgi techniques.

(2) This substance forming the germ-cell determinant of Apanteles is partly gathered into the form of fine granules. Fat solvents and lipin solvents tend to destroy the granular formation, but accentuate and make more chromophil the ground substance of the germ-cell determinant. After fat solvents the latter becomes stringy.

(3) Solvents which disintegrate the yolk-spheres (fat and lecithin) do not remove the germ-cell determinant from the egg.

(4) Tests for fat do not reveal the presence of this substance in the germ-cell determinant.

(5) Fixations which remove the mitochondria from the egg do not remove the germ-cell determinant, while after chrome-osmium fixation, staining methods which do not reveal the mitochondria stain the region of the germ-cell determinant in a basophil manner.

(6) Glycogen methods did not reveal any substance in the determinant which could be identified as glycogen.

(7) The test made for demonstrating the Golgi apparatus (Kopsch's method and that of Cajal) did not bring into evidence any structure which might have been identified as such.

Origin.

(8) The germ-cell determinant originates as a concentrated area at the posterior pole of the young oöcyte. At first it is merely a region of the egg cytoplasm denser than the surrounding, but later it becomes more clearly marked off. No evidence was found supporting the suggestion that the germ-cell determinant contains chromatin. At the time of origin of the determinant the oöcyte nucleus lies at the opposite end of the egg. The secondary or accessory nuclei have no connection with the germ-cell determinant.

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EXPLANATION OF PLATE 9,

Illustrating Mr. J. Bronté Gatenby's paper "On the Germ-Cell Determinant of Parasitic Hymenoptera."

LETTERING.

AR. Archoplasm with mitochondria (?). *CS.* Space in oöcyte free from mitochondria. *F.* Follicle. *FW.* Follicle wall. *FOM.* Outer follicle membrane. *GCD.* Germ-cell determinant. *M.* Mitochondria. *MN.* Nurse-cell mitochondria. *N.* Nucleus. *NCN.* Nurse-cell nucleus. *N.NCN.* Nucleolus of nurse-cell nucleus. *OVW.* Ovariole wall. *PR.OCYTE.* Protoplasm of oöcyte. *SN.* Secondary nuclei. *VA.* Vacuoles (?). *X.* Bodies of uncertain nature, possibly fat. *YD.* Yolk-discs or granules.

In the middle of the plate scales for the figures are given.

FIXATION indicated by the letters: *F.W.A.* Flemming-without-acetic acid. *CH.* Champy. *B.* Bouin's picro-formol acetic. *P.N.* Picro-nitric. *K.* Kopsch.

[All figures drawn from material stained in iron-hæmatoxylin, with the exception of fig. 13. Camera lucida, paper at table level, $\frac{1}{15}$ semi-apochromatic Koritska oil-immersion and compensating eyepieces.]

PLATE 9.

(All figures of Apanteles glomeratus.)

Fig. 1.—Transverse section of upper part of ovariole in region where the cells are as yet undifferentiated into oöcytes and nurse- and follicle-cells. Each cell has a completely differentiated mitochondrial apparatus (*M.*). The ovariole wall is at *OVW.* $\frac{1}{4}$ dilute *F.W.A.*

Fig. 2.—Section of ovariole further down cut longitudinally in region of differentiation of nurse- and follicle cells. The inner cell (*PR.OCYTE*) will be the egg; the other cells will form either follicle- or nurse-cells. In the oöcyte the mitochondria have become grouped around an archoplasm. $\frac{1}{4}$ dilute *F.W.A.*

Fig. 3.—Somewhat older oöcyte showing same cytoplasmic contents, and in addition three or four bodies (X^1. X^3) of doubtful nature, possibly oil or fat. *Ch.*

Fig. 4.—Older oöcyte after formation of follicle (*F.*), which has not all been drawn (see fig. 5). The mitochondria in the oöcyte are granular, and have increased enormously. They are spreading outwards from the nucleus, which at present they embrace. *Ch.*

Fig. 5.—Somewhat later oöcyte; mitochondria becoming fine and stringy, or flocculent. Those of follicle (*F.*) are still granular. *Ch.*

Fig. 6.—Much later stage. Mitochondria now spreading throughout the cytoplasm, but a clear space still exists at *CS.* This stage is just before deutoplasmogenesis sets in. Follicle mitochondria granular. *Ch.*

Figs. 7, 8 and 9.—Three stages in the formation of nurse-cell. In fig. 2, *NCN.*, the nurse-cell mitochondria are few and coarse. In figs. 7, 8 and 9 (smaller scale) they have become very fine, and although they are spread through the cytoplasm, by far the bulk surrounds the nuclear membrane as a dense halo. They never leave this position. *F.W.A.*

Fig. 10.—Transverse section of oöcyte just after beginning of deutoplasmogenesis. The yolk (*YD.*) forms a wall around the periphery of the egg. The flocculent mitochondria have spread throughout the egg. At *SN.* are the secondary nuclei which have mysteriously appeared. Drawn to low scale. *F.W.A.*

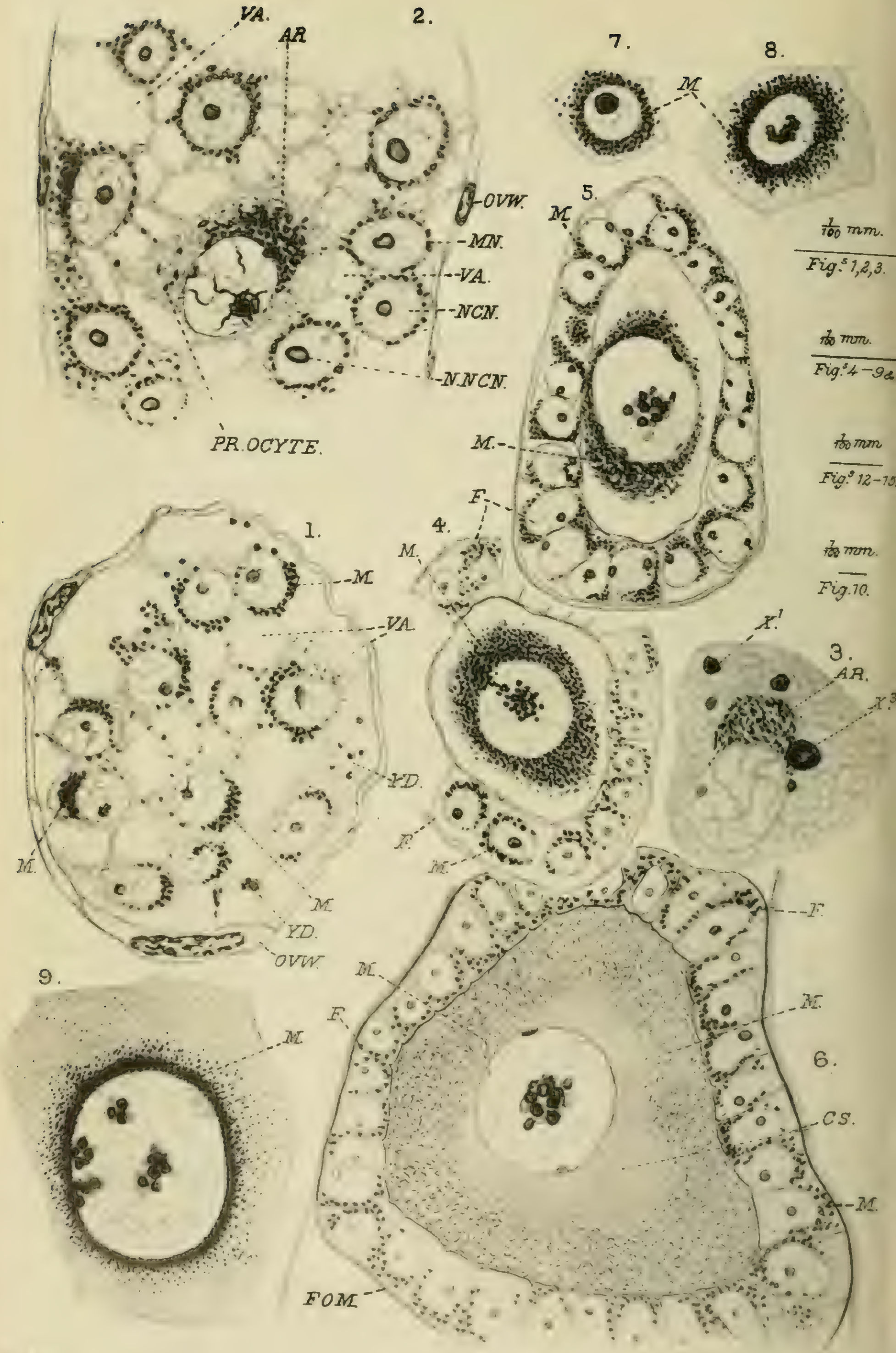
Fig. 11.—The edge of such an egg as that in fig. 10, drawn to much larger scale, showing peripheral yolk (*YD.*), mitochondria throughout as discrete or grouped fibrous units (*M.*). At *SN.* are the secondary nuclei. *F.W.A.*

Fig. 12.—Posterior pole of egg at same stage as that of two previous figures; longitudinal section, showing germ-cell determinant (*GCD.*) as a dark granular mass lying in the centre of the posterior pole of the egg. At the edge of the egg are the yolk-discs (*Y.D.*), the secondary nuclei (*SN.*), and throughout are the mitochondria. *F.W.A.*

Fig. 13.—Posterior end of egg at a little later stage after Kopsch's treatment (OsO_4 for fourteen days), showing the complete extension of the darkened zone, not only on the germ-cell determinant (*GCD.*), but on each side. With the *F.W.A.* method this additional zone of activity is not seen (compare fig. 12). *K.*

Fig. 14.—End of egg at same stage as that of fig. 12, treated for three hours in picro-nitric. Yolk and mitochondria completely dissolved away; only secondary nuclei (*SN.*) and germ-cell determinant show. The latter is more diffuse than with the treatment used for fig. 12. *P.N.*

Fig. 15.—Same region of egg, treated overnight in dilute Bouin (picro-formol-acetic). Yolk shrunken (*YD.*), but determinant still granular. Mitochondria present. *B.*



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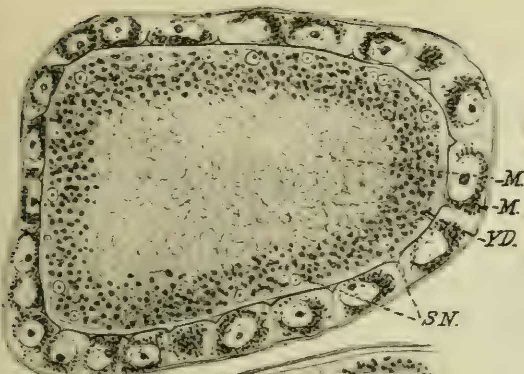

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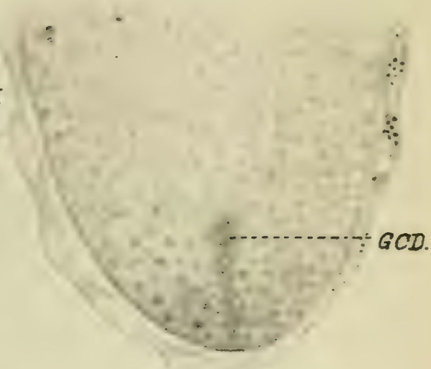

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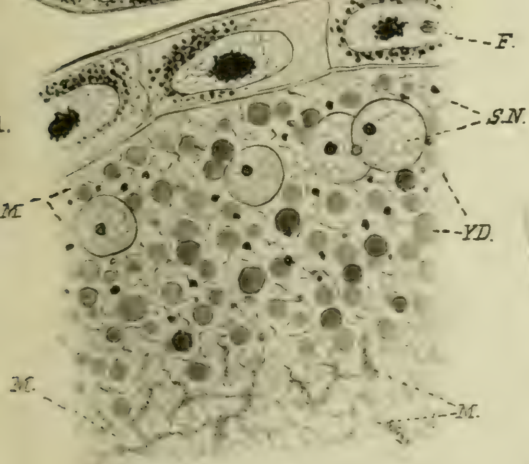

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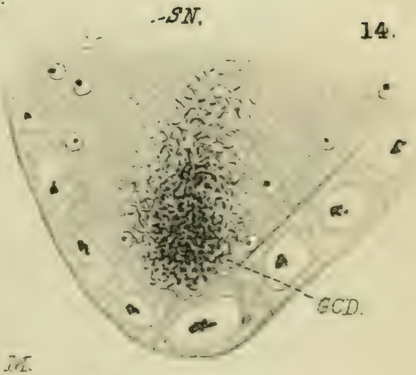

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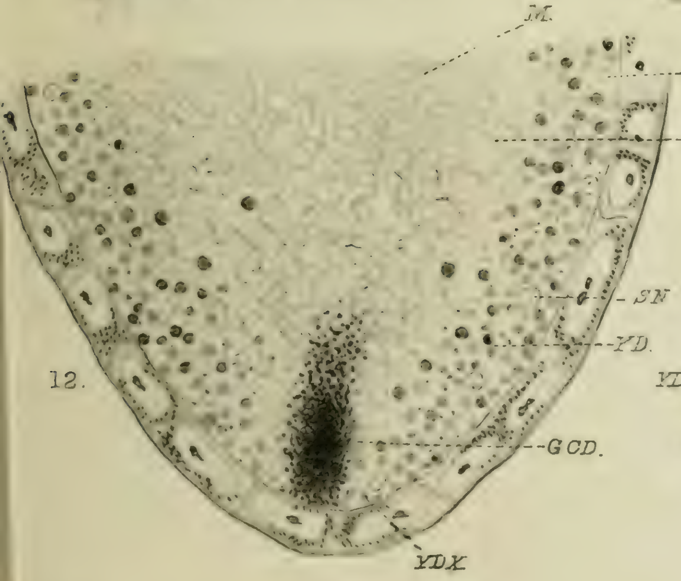

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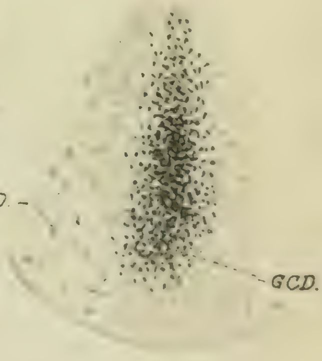

Huth, London

RMINANT IN APANTELES.

The Development of the Starfish Crossaster papposus, Müller and Troschel.

By

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Zoological Department, University College, Dundee.

With Plate 10 and 12 Text-figures.

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The development of Crossaster papposus, though less important than that of starfish having feeding larvæ, nevertheless presents features of great interest, especially in regard to the origin of the primary larval cœlomic cavities, the madreporic vesicle, the extra rays, the skeleton, and the general modifications due to yolkiness of the egg. Crossaster papposus much resembles Solaster endeca in development, and accordingly I may be allowed to refer to a former paper on the latter (**7**) for details and illustrations which do not call for repetition (see, e.g., p. 3 and p. 34).

The Crossasters and Solasters are members of the Solasteridæ, a family of Cryptozonia in the classification of Sladen (**23**), and of Spinulosæ in that of Perrier (**22**). In this family the abactinal skeleton is more or less regularly reticulate, many of the plates bearing paxilliform groups of spines; single interbrachial septa are present; there are no pedicellariæ and no supradermal membranes; actinal intermediate plates occur, more or less developed; the adambulacral armature is pectinate, a transverse series being present in all, and also a longitudinal in some.

The two common British species Crossaster papposus and Solaster endeca show the following differences: In the former the abactinal recticulum is somewhat widely meshed, and the groups of paxillæ are spread well apart, each having relatively few but large spines; the papules are numerous; the number of rays is ± 12 (**18**); the colour of the abactinal surface is a brighter or darker red, often shading into orange or yellow, especially on the rays. In Solaster endeca, on the other hand, the abactinal reticulum is somewhat narrow and irregular, the nodal plates being often stellate in shape; the paxillæ are small and closely crowded; the papules are few, the number of rays is ± 9 (**18**); the colour of the abactinal surface is a rich purple.

Among the resemblances between Crossaster papposus and Solaster endeca which are of systematic or structural importance and have not been indicated above we may note the following: The adambulacral armature is in two series, one at right angles to the other; no actinal intermediate plates occur along the rays, and there is one series of well-developed marginal paxillæ (**23**, p. 442). The madreporite is single and may carry spines; the tube-feet are biserially arranged; a Polian vesicle and two Tiedemann's bodies are present in all the interradii except in the madreporic interradius where one of the latter is wanting; the anus is in interradius V/VI,[1] no matter what may be the number of rays; the rectal cæca are well developed and of similar form; the cilia everwhere exhibit similar activities.

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[1] The madreporite is reckoned as lying in interradius I/II, ray I being on its sinistral side. Throughout this paper "dextral" and "sinistral" indicate respectively the sides towards which, or away from which, the hands of a watch would appear to move on a starfish disc as viewed aborally.

Crossaster and Solaster have evidently long been separated from one another since both are widely distributed. Solaster, however, has the greater range, both geographically and bathymetrically, and as it has fewer rays and includes a somewhat greater number of species, we may conclude that it is probably the more primitive of the two genera. The earliest known Solaster is a thirty-rayed form (S. moretonis, Forbes) occurring in the oolite (**5**).

The five-rayed Cribrella, though it is placed in another Cryptozonate family, the Echinasteridæ (**23**), agrees with Solaster in its chief characters excepting the number of rays. In development Cribella (Masterman, **19**, 1902) and Solaster (Gemmill, **7**, 1912) show close parallelism excepting as regards the madreporic vesicle (p. 168).

Early Development up to Gastrulation.

The gonads are bunches of short, unbranched, lobulated or very simply branched tubes, reddish or orange in colour, paler in the males, confined to the disc, and not extending out into the arms.

(For details regarding genital ducts, growth of ova, and relations to hæmal and perihæmal systems see **6**, pp. 184–5, and **7**, pp. 4–9.)

The season of ripeness in the Firth of Clyde lasts from about the beginning of March to the end of the first week in April. Each starfish may spawn several times during this period, at intervals of two to ten days. The presence of other spawning individuals acts as a stimulant, and "epidemics" of spawning occur in tanks containing numerous starfish. A full-sized female may shed more than two thousand ova at a time, and probably more than six thousand altogether. The brooding habit is absent. There is no invariable spawning position. Commonly, however, spawning starfish will be found bunched up under water and adherent only by the outer portions of their rays, or else right at the surface with half of the disc and arms detached and extended outwards. Occasionally they may be observed spawning in the ordinary extended position, motionless under the water.

The ova, which are a little smaller than those of S. endeca, measure about ·8 mm. in diameter, being rather wider in equatorial than in vertical measurement. They float, the lower pole being rather lighter in weight and paler in colour than the upper one. There is more variety of colour than in the case of S. endeca, the general tint in ova from different starfish varying from reddish-orange to terra-cotta or clay. The polar bodies emerge from the middle of the upper pole. Maturation is in progress as the eggs are being shed and fertilisation is followed by formation of a membrane of fertilisation.

Segmentation is total and equal, the first two cleavages being vertical and at right angles to one another, while the third is equatorial. Continued cleavage produces a practically solid mass of small cells of which more than a hundred can be counted in a single section. Blastula formation takes place by egression (see Masterman, **19**, p. 380) of the more centrally placed cells along lines which show up as grooves on the surface. During this process the cell walls disappear in great part, except just at the surface, so that the interior is almost a syncytium. The egression grooves are at first numerous, and their arrangement recalls that of the surface furrows in the later segmentation stages. Gradually the grooves become fewer and simpler until resemblance to a sixteen-celled, an eight-celled, a four-celled, or even a two-celled stage is reached. The solid morula is now converted into a hollow single-layered blastula. Its wall increases in thickness as the folds smooth out, only losing the last of these as gastrulation proceeds.

Gastrulation begins in the middle of the under-surface, to all appearance by deepening of an egression groove in this region, or of two such grooves crossing one another. The gastrula opening is therefore at first usually elongated, but sometimes cruciform, or tri-radiate. As the involution deepens, all other superficial markings are smoothed out, and the gastrula opening becomes circular. The transition between the paler cells of the under-pole of the egg and the more highly-coloured cells of the upper pole is to be found a short distance out from the lip of the blastopore all round. The gastrula is now somewhat flattened on the under side, its vertical being a little less than its equatorial diameter.

Cilia appear on the surface during blastula formation while the egg is still enclosed within the membrane of fertilisation. This membrane becomes lost about the time when gastrulation begins. The young gastrulæ swarm slowly about, at first somewhat irregularly, but afterwards rotating on their vertical axes with slight epicyclic progression in a direction corresponding with the rotation. The rotation may be dextral or sinistral as viewed from above, and not infrequently reverses itself. In section the gastrula is found to be two-layered, each layer being made up of elongated columnar cells with cilia on their free surfaces. During blastula formation, yolk-granules are often extruded from the surface, and a quantity of these may be swept into the archenteron, which, however, at this stage is otherwise empty as a rule, the formation of "hypenchyme" being either absent altogether or very much less well-marked than has been described by Masterman in the case of Cribrella (**19**).

External Changes up to and After Fixation.

Early Larva up to Fixation.—The gastrula elongates to about twice its former vertical measurement, the middle of the upper surface becoming the anterior extremity of the larva. A depression forms on what will be the ventral side of the larva near the junction of its second and third fifths. Rudiments of the three brachia then grow out, one in front of and the other two to right and left of, and slightly behind, the middle of this depression, where the rudiment of the sucker now appears. The portion of the larva which carries the brachia and the sucker may be called the præoral lobe. The posterior portion or body of the larva has meantime become flattened out in the sagittal plane, and the blastopore has gradually become smaller. As a rule it still retains its original position in the centre of the posterior end of the larva, although in a good many cases it becomes pressed slightly ventrally and to the right owing to prominence of its left dorsal margin. Otherwise the larvæ exhibit till now perfect bilateral symmetry.

A shallow ectodermal depression, at the bottom of which the hydroporic opening forms, appears on the right side of the body of the larva anteriorly some distance from the dorsal edge. The next important features to be noted are closure of the blastoporic opening, increase in length of the brachia, greater prominence of the sucker, sharper division of the larva into præoral lobe and body, the last-named portion becoming more disc-like through flattening from side to side.

As regards movements, at first the larvæ, being still rather lighter than water, remain in a vertical or slanting position with the anterior end uppermost, rotating clockwise as viewed from the anterior end, and progressing slowly with this end in advance. Reversals of the direction of rotation and progression may occur for short periods. Later, the larvæ (Pl. 10, figs. 1–4), becoming relatively heavier, rise or sink in the water, according to the activity of their ciliary action. The brachia now show movements of shortening and lengthening, closing together, and divarication. Their ends are glandular and slightly adhesive, enabling the larva to attach itself loosely to smooth surfaces.

Fixation by the sucker occurs about the nineteenth day. The larvæ take first hold by the tips of the brachia, which then become widely separated and shortened by muscular action, while at the same time the sucker is pushed out. The sucker adheres at first by muscular action, but afterwards by throwing out a thin layer of cement. A very important external change is occurring, namely, flexion and torsion of the præoral lobe on the body or disc, with the left side of which this lobe becomes gradually incorporated (Pl. 10, figs. 5–7). The flexion is a leftward one, and when completed may be counted as being through two right angles, since it ultimately flattens out the anterior end of the larva on the left side of the disc. The torsion takes place dextrally as viewed from the anterior end of the larva, through an angle of 75° or thereby. The hydrocœle lobes appear externally as swellings very shortly after fixation has been effected (Pl. 10, fig. 6). Lobes III and IV usually show up first, then II and V, then VI, and lastly I. This is slightly different from the sequence in which the radial pouches first make their appearance internally on the wall of the hydrocœle (see p. 163).

The flexion and torsion of the præoral lobe produce a marked notch on the margin of the disc in the junctional region on its (larval) dorsal side. This is the so-called aboral brachiolarian notch. It afterwards becomes gradually filled out, forming interradius I/II—the madreporic inter-radius. Shallow indentations also appear on the margin of the disc, indicating succeeding interradii, namely, II/III to V/VI. There is still a segment of the disc between V/VI and I/II, amounting to rather less than a third of a circle, which has as yet no notches, but in which the remaining arm-rudiments will afterwards form.

Hydrocœle lobe VII next appears beyond VI, on the sinistral[1] side of the latter, and much further back in differentiation. The next lobe to become evident is XII, which arises close to I, and on its dextral[1] side. Lobe VIII now makes its appearance sinistral to VII, and shortly thereafter XI dextral to XII. Lobe IX (sinistral to VIII) is the next to show up, and X the last in the small interspace still intervening between IX and XI. Meantime the general mass of the præoral lobe, as well as the brachia, and finally, the sucker, have been disappearing, and the last remains of the sucker are to be found opposite lobe X or in interradius IX/X, just outside the completed hydrocœle ring (Pl. 10, figs. 8–9). At the time of their first appearance the hydrocœle lobes are blunt swellings; then they become trilobate through differentiation of the proximal pair of sucker feet and of the terminal tentacle. Succeeding pairs of sucker feet appear between the terminal tentacle and the latest formed pair. About the twenty-eighth day the proximal pairs of sucker feet

[1] See footnote on p. 156.

on the first six hydrocœle lobes begin to be functional. The young starfish may now shift position while still remaining anchored by the sucker, the stalk of which can stretch out a length of over 1 mm. Similar movements occur at a corresponding stage in the case of A. rubens, but whereas in the latter starfish the sucker ultimately separates off and is left behind, in Solaster this structure, after becoming smaller, loosens its hold, and is absorbed. The loosening usually takes place before lobes IX, X and XI have become clearly marked out. In the course of further growth the formation of interradial notches between the later-formed rays is gradually effected. Notches VI/VII and VII/VIII appear first, then XII/I, the remaining notches, viz. VIII/IX, XI/X and IX/X being the last to appear. By this time notch I/II, i.e. the brachiolarian notch, has filled out so that it is no deeper than the rest. It will be remembered that arm-rudiment I is formed from the (larval) ventral lip of the notch in question.

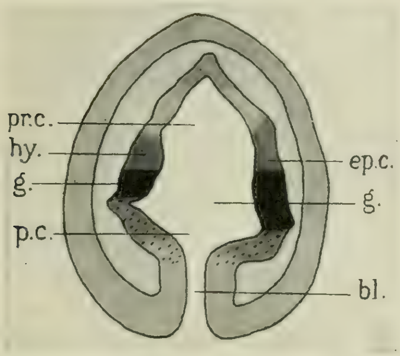
Slight prominences containing developing spines begin to appear about the twenty-second day all over the aboral surface, including the interradial notches and the sides of the aboral arm-rudiments (see under "Skeleton," p. 178). The spines develop latest on the part of the disc where the last formed arm rudiments are gradually becoming marked out (Pl. 10, fig. 8).

The mouth appears about the thirty-eighth day, characteristically in the form of a tri-radiate fissure, the angles of which point more or less directly towards interradii XII/I, III/IV, and VII/VIII (Pl. 10, fig. 9). The anus appears much later—about the end of the seventh week.

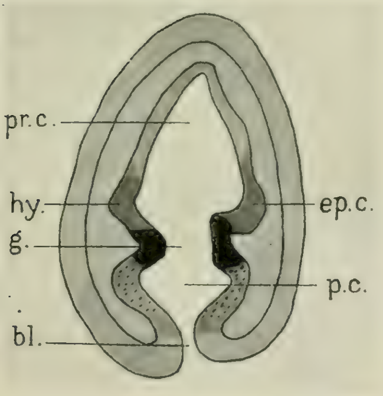
Development of Cavities and Organs.

At first the archenteron follows the shape of the elongating larva, but about the seventh day begins to show constrictions marking off (a) anterior, (b) middle and (a) posterior regions, without there being any indication of such a division on the external surface of the larva (Text-figs. 1–4). These regions are respectively (a) the anterior larval cœlomic cavity, (b) the enteron, and (c) the posterior larval cœlomic cavity,

TEXT-FIG. 1.



TEXT-FIG. 2.



TEXT-FIGS. 1–2.—See explanation on p. 165.

and it will be now convenient to follow out the development of each, noting meantime that mesenchyme arises abundantly at this stage by budding from the walls of the anterior and posterior cavities, but chiefly of the latter.

Anterior Larval Cœlomic Cavity.—This cavity extends (1) into the præoral lobe and the brachia and (2) backwards to right and left of the enteron, forming right and left lateral diverticula, and it also sends from its posterodorsal portion a pocket to the right, which becomes the hydroporic canal, the inner end of the latter being, to begin with, approximately in the middle line. The outer end of this canal meets and fuses with the bottom of a slight pit on the right side of the larva dorsally, giving rise to the hydroporic opening. The following derivatives of the anterior larval cœlomic cavity now fall to be described: cavity of præoral lobe, hydrocœle, epigastric cœlom, axial sinus, axial organ, stone-canal, and internal oral circular sinus.

Cavity of Præoral Lobe.—The cavity of the præoral lobe gets obliterated as the lobe itself is reduced. The attaching stalk has become solid by the time the ring-canal of the hydrocœle is completed, and accordingly we cannot say that the hydrocœle ever encircles the stalk-cavity. However, the central portion of the anterior cœlom from which the stalk-cavity came off is still relatively large at the stage we are considering, and lies within the circle of the completed hydrocœle ring.

Hydrocœle.—The hydrocœle pouches appear as outpocketings of the left lateral diverticulum of the anterior cœlom. Pouches I–V are formed at much the same time, I being, perhaps, a little delayed; VI comes immediately thereafter sinistral[1] to V; then, more slowly, VII sinistral to VI; then XII dextral[1] to I; then VIII sinistral to VII; then XI dextral to XII; then IX sinistral to VIII; and finally X in the interspace remaining between XI and IX. It will be remembered from p. 161 that the external swelling for pouch I was relatively somewhat later in appearing, probably owing to the imprisonment of this swelling in the corner to which the præoral structures are being retracted. The hydrocœle ring-canal gets nipped off from the bottom of the left lateral diverticulum earliest in the

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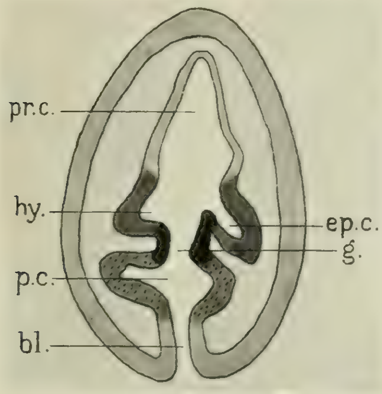
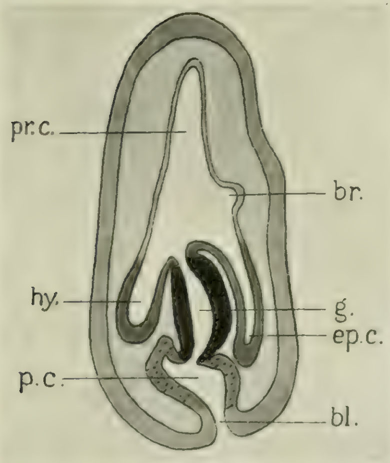
[1] See footnote on p. 156.

TEXT-FIG. 3.



TEXT-FIG. 4.



Text-figs. 1–4.—Optical sections of Crossaster larvæ (7–8 days), to illustrate subdivision of archenteron into gut and primary larval cœlomic cavities. The smaller size of the left wall of the enteric isthmus will be noted all through, and at stage shown in Text-fig. 4 a greater backward extension of the epigastric cœlom than of the hydrocœle. *bl.* Blastopore. *br.* Cavity of an outgrowing lateral brachium. *ep. c.* Epigastric cœlom. *g.* Enteron. *hy.* Hydrocœle. *p. c.* Posterior cœlom. *pr. c.* Præoral cœlom.

region of pouches II–IV. This canal is at first crescent-shaped and open at either end, but shortly after fixation, by extension of the nipping-off process, its dorsal and ventral horns end blindly. The blind ends have now to push their way round from either side so as to meet, and by uniting complete the hydrocœle ring. The completion takes place in interradius IX/X, rather nearer X than IX. Pouches VI to IX have arisen from the ventral horn of the hydrocœle crescent and pouches XII–X from the dorsal horn. At first the hydrocœle crescent is a segment of a relatively small circle. Later, the area enclosed becomes much larger through incorporation of præoral lobe epiderm with the body in this region. This increase in size provides room for the formation of the mouth. The sucker and the brachia disappear by histolytic absorption.

Epigastric Cœlom.—The right lateral diverticulum of the anterior larval cœlomic cavity becomes cut off by constriction from the præoral cœlom, and since it gives rise to the adult epigastric cœlom is to be looked on as equivalent to a right posterior cœlom (Asterina (**13**), Asterias rubens (**7**), Porania (**9**), and cf. p. 180). The constriction is a result of (1) the flexion and torsion of præoral lobe already described, and (2) the growth of the dorsal and ventral horns of the posterior cœlom (p. 174). The intestine and rectum grow up (sixth week) as an aboral-ward pouch between the layers of the mesentery separating the epigastric from the hypogastric cœloms in interradius V/VI near the middle of the aboral surface. This mesentery afterwards becomes broken up to form the aboral ligaments of the stomach. A bilobed pouch from the epigastric cœlom extends outwards in each ray above the developing radial cæca (p. 177), its walls forming the aboral or suspensory ligaments of these cæca.

Axial Sinus.—The axial sinus is the remains of the central portion of the anterior cœlomic cavity after (1) the præoral lobe portion disappears, (2) the hydrocœle ring-canal is nipped off from the left lateral diverticulum, and (3) the internal oral perihæmal ring is (see below) reduced to its proper size. The hydroporic canal now opens internally to the right or aboral side of the former larval sagittal plane.

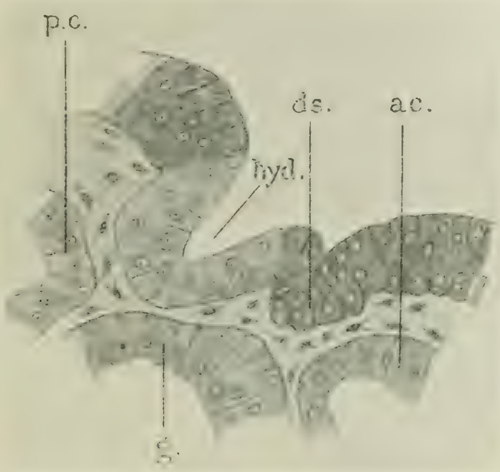
Stone-canal.—As in Solaster endeca, the stone-canal appears to be formed partly as an outgrowth from the hydrocœle groove in interradius I/II and partly as a furrow in the wall of the axial sinus. Furrow and outgrowth establish continuity with one another and the furrow becomes roofed in except at its aboral extremity near the internal opening of the hydroporic canal, where it opens into the axial sinus. For an account of the later development and adult structure of the pore-canal system and madreporite see (**8**).

Axial Organ.—The axial organ begins during early metamorphosis as a cell thickening, soon rising into a fold, of the wall of the axial sinus, commencing below the madreporic vesicle and continued orally into a somewhat prominent crescent of spongy tissue in the still spacious (see below) portion of the internal oral perihæmal ring opposite the stalk. (This crescent, afterwards becoming smaller and extending round the mouth, forms the oral hæmal ring.) The fold of the axial organ contains (1) spongy channels continuous with those which are being invaginated from below into the madreporic vesicle (p. 168); (2) cells derived from the lining of the axial sinus; (3) cells derived from the floor of the madreporic vesicle and its oral-ward pocket, some connected with fibres running chiefly in the longitudinal axis of the fold. A little later than (1) and (2) the dorsal horn of the posterior cœlom provides a well-marked ingrowth (of primitive germ-cells—MacBride, Asterina, Amphiura, etc.) just below the madreporic vesicle.

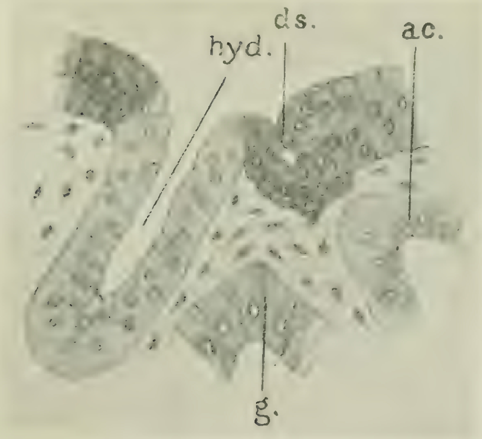
Internal Oral Perihæmal Ring.—This ring (internal oral circular sinus) is a derivative of the central portion of the anterior larval cœlomic cavity and of that portion of the left lateral diverticulum which remains after the hydrocœle canal is nipped off. In the formation of the mouth, epiblast and hypoblast become continuous across these remainders, which are not, however, entirely obliterated. What is left of them lies around the mouth and forms the internal oral sinus, being spacious at first in the region of the stalk, but for a time obliterated just opposite to that region, so that we can describe the sinus in question as being in a sense completed by outgrowth from the axial sinus. In the adult, as in the larva, it communicates with the axial sinus.

Dorsal Sac or Madreporic Vesicle (see also p. 181).—Contrary to all expectation the madreporic vesicle has been found to arise from epiblast (Text-figs. 5–6), sometimes as a solid ingrowth of cells, afterwards becoming separated off and acquiring a lumen, and sometimes by the formation of a pit, the bottom of which gradually becomes constricted off from its parent surface (Text-figs. 7–8). Its place of origin is immediately ventral to the hydroporic opening, and close to the junction of the epiblast with the enterocœlic wall of the hydroporic canal. Normally it begins to form just after the hydroporic opening has been established, but its rudiment is sometimes recognisable prior to this event. At first the vesicle wall is made up of columnar or cubical cells. These afterwards become flattened. Towards the end of metamorphosis the madreporic vesicle is found to have sent down a rather long, narrow oral-ward pocket into the upper end of the axial organ. Beneath the vesicle on the side looking towards the pore-canal a vacuolated space appears (thirteen to sixteen days) in the mesenchyme. Prior to metamorphosis this space can be recognised as extending (Text-fig. 10) at one part into the tissue of the future aboral body-wall, and at another over the aboral wall of the stomach, while a later extension can be noted down the wall of the axial sinus in the line of the developing axial organ. The main space is spongy, being traversed by fibrillæ, and the spongy tissue is invaginated at metamorphosis into the madreporic vesicle, forming the so-called head-process of the axial organ, which appears to be homologous with the "heart" of Balanoglossus. Tissue opacity precludes observations on the question of pulsation in the larva, but a dissected-out "heart" in the adult shows faint sub-rhythmic contractions (**8**, p. 272). In later growth the invagination of the "heart"

TEXT-FIG. 5.

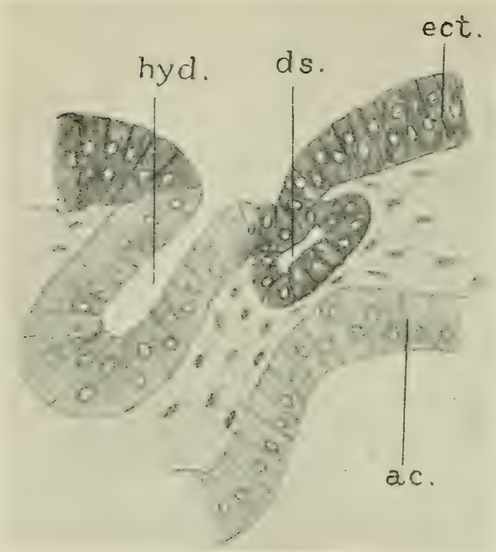


TEXT-FIG. 6.



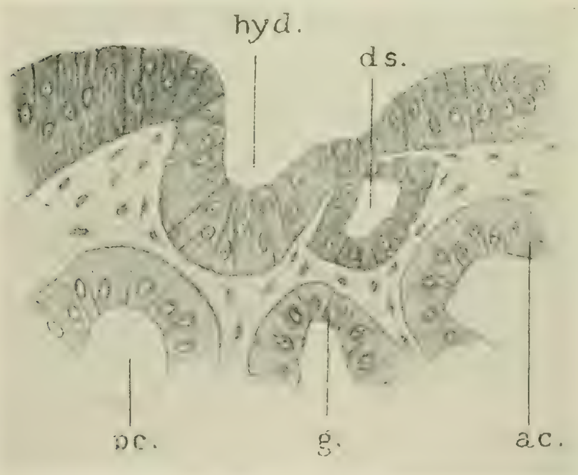
Text-figs. 5–6.—Sections across hydroporic region of Crossaster larvæ, 12 days old, drawn as viewed from posterior end of larvæ. The layers and nuclei were mapped out with the camera lucida. At this stage the dorsal sac is a thickening or an involution of epiblast immediately ventral to the junction of the latter with the wall of the hydroporic canal. *ac.* Anterior cœlom. *ds.* Dorsal sac. *g.* Enteron. *hyd.* Hydroporic canal. *pc.* Posterior cœlom.

Text-fig. 7.



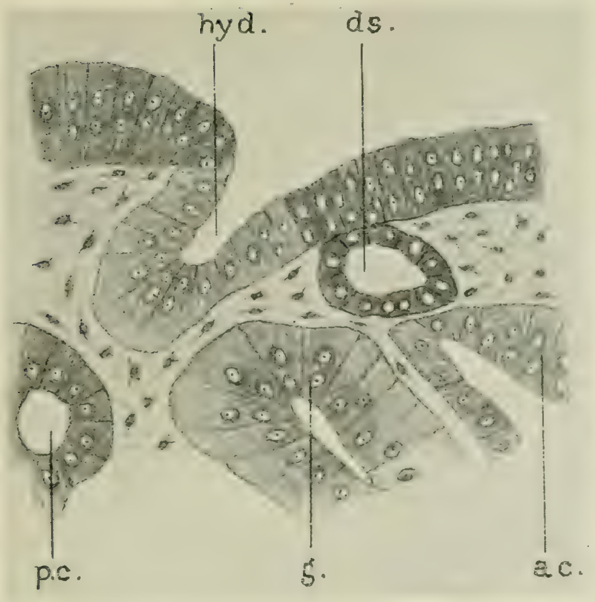
The dorsal sac is becoming constricted off from the epiblast and has a minute lumen. (See under Text-figs. 5–6, p. 169.)

Text-fig. 8.



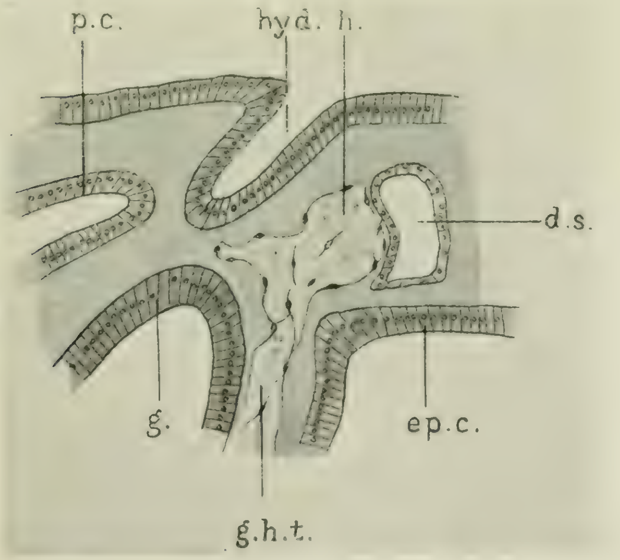
The sac is increasing in size (13–14 days) and is almost completely separated off from the epiblast, the surface of which, as in Text-fig. 7, still shows a slight dimple marking where the involution of the sac first took place. (See under Text-figs. 5–6, p. 169.)

TEXT-FIG. 9.



The sac is now (15 days) completely separated off from the epiblast, but is still relatively thick-walled. (See under Text-figs. 5–6, p. 169.)

TEXT-FIG. 10 (semi-diagrammatic).



The sac has now migrated deeper (16–17 days), is thinner-walled, and shows beside it a spongy space, the future "heart" (*h*), channels from which are seen extending on the future aboral aspect of the gut-wall. (See under Text-figs. 5–6, p. 169.)

into the madreporic vesicle can be recognised as being along a line, at the two extremities of which are the communications of the "heart" respectively (1) with the body of the axial organ, and (2) with the aboral body-wall spaces, the former being on the deep side axially and the latter on the superficial side. The two extremities retain their proper connections, but the portion between them loses its attaching mesentery ("mesocardium") within the madreporic vesicle, as occurs in the case of a developing vertebrate heart-tube within the pericardial cavity.

The relations of the central portions of the hæmal system are illustrated in Text-figs. 11–12, and reference may be made for further details and for a comparison with the hæmal system of Balanoglossus to **8**, pp. 271 and 278, and to 'Quart. Journ. Micr. Sci.,' 1919, p. 537. Reasons are there given for instituting a homology between the axial organ in Echinoderms and the left collar vessel and the left part of the glomerulus in Balanoglossus. The vessel in question is a rete mirabile (Spengel, **25**, p. 753), which lies within a fold of the left collar cœlomic wall.

Madreporite.—The relations of pore-canals, axial sinus, etc., in the adult madreporites of Asterias rubens (**8**) and Solaster endeca (**6**) have already been described by the author. In the last-named star-fish the ampulla of the "stone-canal" is represented by a sac with thick glandular walls completely invaginated into the madreporic vesicle, and connected by a long, narrow neck with the collecting spaces of the pore-canal system. Light is thrown on the development of such an ampulla by intermediate Crossaster growth stages where (e.g. in specimens from $\frac{1}{2}$ in. to 1 in. across) the ampulla is a thick-walled wide-necked cavity bulging into the madreporic vesicle (Text-fig. 12). In Asterias rubens the ampulla is relatively large and lies directly over the madreporic vesicle, from which it is separated by a thin membrane.

The ampulla has probably a hydrostatic function (**8**, p. 272), and permits changes of volume on the part of the "heart"

Text-fig. 11.

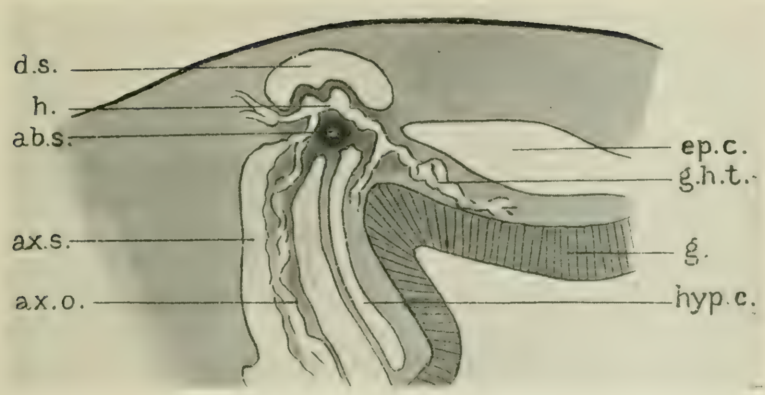


Diagram illustrating relations of heart towards end of metamorphosis, showing its communications (the future gastric hæmal tufts) with the stomach-wall, and also with the axial organ and the aboral body-wall. (See under Text-fig. 12.)

Text-fig. 12.

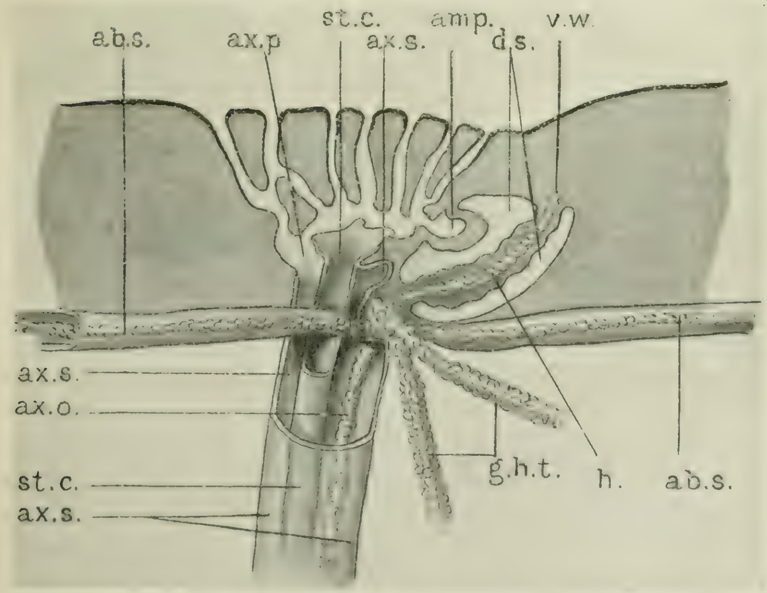


Diagram to illustrate relations of pore-canal system, axial sinus, etc., madreporic vesicle, "heart," and principal hæmal vessels in a young Crossaster, 25 mm. across the disc (see p. 172). The madreporite, the madreporic vesicle, the ampulla, and the upper parts of the stone-canal and axial sinus are seen in vertical section as from somewhere near the centre of the disc. *ab. s.* Aboral sinus. *amp.* Ampulla of stone-canal. *ax. o.* Axial organ. *ax. p.* Main opening of axial sinus into pore-canal system. *ax. s.* Axial sinus. *ds.* Dorsal sac or madreporic vesicle. *g.h.t.* Gastric hæmal tufts. *h.* "Heart." *st. c.* Stone-canal. *v. w.* Communication of "heart" with spaces in aboral body-wall.

(head-process of axial organ) within the madreporic vesicle, this vesicle being surrounded by rigid tissue except where it abuts against the ampulla.

The Larval Posterior Cœlomic Cavity.—When first recognisable this cœlom is a rounded cavity, which sometimes lies symmetrically at the posterior end of the larva, but sometimes appears swollen dorsally and to the left, causing the prominence of the left dorsal part of the blastoporic lip referred to on p. 159. As the body of the larva becomes flattened laterally the posterior cœlom grows out dorsally and ventrally, giving rise to dorsal and ventral horns, the former of which at first keeps slightly to the left of the middle line. The dorsal horn grows forward till it is arrested by the axial sinus portion of the anterior cœlom. At this stage it often shows bifidity, the left tip being destined to form perihæmal pouch I/II, and the right one pharyngeal pocket I/II and the cœlom within arm-rudiment II. The ventral horn extends first in a forward and then in a dorsal direction until it also is arrested by the anterior cœlom. The tip of this horn lies in the ventral lip of the aboral brachiolarian notch, and when the notch in question becomes reduced to form interradius I/II, gives rise to the cœlom within arm-rudiment I. Accordingly, while hydrocœle pouch I is derived from the larval dorsal, arm-rudiment I is derived from the larval ventral side of the brachiolarian notch. In its growth forward the ventral horn passes slightly to the right of the middle line and its tip also sometimes shows temporary bifidity. The principal derivatives of the posterior cœlom are the hypogastric cœlom, the pharyngeal or perioral cœlom, the external oral perihæmal pockets, the genital rachis, and the aboral perihæmal sinus.

Hypogastric Cœlomic Cavity.—This large cavity is derived wholly from the posterior cœlom and its dorsal and ventral horns. During and after metamorphosis it extends orally and aborally, thus covering a portion of the enteron both on the oral and aboral sides. The layers between it and the epigastric cœlom form the circular epigastric mesentery, which ultimately becomes broken up into ligamentous strands except above the radial cæca. Outgrowths from the main cavity give rise to the cavities within the arm-rudiments, that of arm-rudiment II being derived from the tip of the dorsal, and that of arm-rudiment I from the tip of the ventral horn. During, and for some time after, metamorphosis, the dorsal and ventral horns are separated by the axial sinus, which abuts against the enteron wall. Later, however, the two horns communicate freely between axial sinus and enteron wall, owing chiefly to extension of the dorsal horn in this region. The two gastric hæmal strands are specialised bands belonging to the series derived from the circular epigastric mesentery (p. 166).

Perioral or Pharyngeal Cœlom.—This cœlom arises in the form of pockets from the posterior cœlom, coming off internal to the hydrocœle ring, and pushing into the tissue between this ring and the gut. Pockets III/IV and IV/V arise earliest, then II/III, V/VI, VI/VII, XII/I, I/II, VII/VIII, XII/XI, VIII/IX, XI/X, and IX/X. The first-formed pockets (I–VI) are completely separated off at a stage when the later ones (VIII/IX and X/XI) are in course of formation and the last one (IX/I) has not yet appeared. Pouch I/II arises from near the tip of the dorsal, and pouch XII/I from near the tip of the ventral horn; after their separation the pockets extend towards one another, and ultimately fuse to form a continuous cavity round the position of the future mouth. Their stalks of origin become obliterated with the exception that the first of them, i.e. that in interradius I/II, does not appear to close. When the fusion of the pockets is complete, the resulting pharyngeal cavity is separated from the main hypogastric cavity by a circular mesentery, except in interradius I/II. This sheet becomes perforated first interradially and then radially, giving rise to the various ligaments connecting the oral aspect of the stomach with the body-wall.

External Oral Perihæmal Ring.—This ring arises in the form of interradial pockets, all of which take origin from the posterior cœlom, and, growing out external to the hydrocœle ring, interdigitate with the hydrocœle pouches. They appear several days later than the pouches between which they lie and in similar sequence. Thus, those in interradii I/II to V/VI appear practically together, III/IV and IV/V being a little in advance. Then in order come VI/VII, XII/I, VII/VIII, XII/XI, VIII/IX, X/XI and IX/X, as in the case of the pockets for the pharyngeal cœlom. I/II, II/III, etc., arise from the dorsal horn, and XII/I, XI/XII, etc., from the ventral horn of the posterior cœlom. As the radial canals of the hydrocœle grow out, each perihæmal pocket sends a prolongation along the adjacent sides of the two radial canals bounding the interradius in which it lies, as MacBride first pointed out in the case of Asterina (**13**, p. 560). As in Solaster (**6**, p. 188) and Asterias (**8**, p. 260) each pocket also sends an interradial branch aborally into the tissue of the interbrachial septum.

Genital Rachis and Aboral Perihæmal Ring.—So far as I can make out the development of these two structures occurs in the manner described by MacBride for Asterina (**13**), viz. by the formation of a pocket pushing out from the dorsal horn of the posterior cœlom just below the madreporic vesicle, and afterwards extending horizontally round the disc and sending two branches outwards in each interradius. I do not find, however, that there is a growth of recognisable germ-cells down the length of the axial organ.

The Larval Middle Cavity or Enteron.—At first the enteron is simply an isthmus between the anterior and posterior cœloms, compressed from side to side so as to form a short slit-like passage. The dorsal extremity of the slit is a little to the left of the middle line and the ventral extremity a little to the right. The right wall of the slit is rather larger than the left and is slightly pouched to the right. The opening between the enteron and the posterior cœlom becomes closed about the eighth day, its last traces being found nearer the ventral than the dorsal end of the slit and looking slightly to the right. The opening between enteron and anterior cœlom does not close till a day or two later. The last trace of this opening is found near the dorsal end of the slit, and looks leftward owing to the greater size and prominence of the right enteron wall in this region. After separation the enteron expands slowly, becoming, in the first place, bluntly gibbous in shape, with dorsal and ventral horns which fit within the crescent formed by the posterior or hypogastric cœlom. During metamorphosis it increases in size and thickness, and a very active transference of yolk-granules from the other tissues, particularly from the parts undergoing reduction, takes place into the cells forming its walls, which for a time become syncytial. Blunt expansions next appear opposite the rays, and these will be recognisable afterwards as the radial dilations of the gastric portion of the stomach. At the same time a pyloric sac region becomes marked out aborally, and from this sac the pyloric or radial cæca begin to grow outwards. Both kinds of outgrowths appear first in the earliest-formed rays, and subsequently in the other rays, according to the sequence in which these became differentiated. We have to note that the cæca for ray I arise from what was the ventral horn of the gut during its former gibbous stage.

The œsophagus takes origin (sixth to seventh week) on the (larval left) adult actinal aspect, as an outgrowth of the enteron which bridges the central part of the anterior cœlom and the remains of the left lateral diverticulum of this cœlom, and fuses with the left body-wall in the middle of the area enclosed by the hydrocœle crescent. The contingent layers become reduced and then absorbed. There is no stomodæal invagination. Usually, though not always, the mouth opening is at first tri-radiate, the angles pointing more or less in the direction of rays III/IV, VII/VIII, and XI/XII.

The intestine takes origin excentrically in interradius V VI as an outgrowth from the roof of the pyloric sac between the layers of the circular epigastric mesentery. The outgrowth expands at its extremity to form the rectal sac, which projects chiefly into the hypogastric cœlom. The rectum grows out from this sac in interradius V/VI (see **6**, p. 180), continuing the line of the intestine. It gradually burrows through the aboral body-wall in the same inter-radius, and meeting with a shallow depression from outside forms the anus, which, however, is unprovided with a proctodæum.

SKELETON.

The aboral skeletal plates arise as in Solaster endeca (**7**, p. 41) in the form of scattered calcifications, without definite radial or inter-radial arrangement, except that groups of two or more are recognisable from an early stage in the position of the future terminals. As the disc increases in size, additional calcifications appear between the original ones. In the end the earlier-formed plates become nodal points in the skeletal reticulum, and each develops on its superficial aspect a rounded boss or hump for the support of a group of spines. An important point to note is that in each ray the adult terminal arises by the fusion of several (usually five) originally separate calcifications placed at the tip and just at the sides of the tip of the ray. The same process occurs in Solaster endeca (**7**) and in Cribrella (**10**). So far as I know, this is the first ascertained instance among Echinoderms in which a primary skeletal plate takes origin by the union of earlier scattered calcifications. It is altogether likely that in primitive Echinoderms the skeletal plates were numerous, small, and without definite arrangement, and we are probably justified in inferring that the primary plates which appear radially and interradially in the development of such starfish as Asterina gibbosa and Asterias rubens are the ontogenetic representatives of groups of ancestral plates. This circumstance may help us to realise how far any particular form must have travelled towards specialisation by the time it exhibits quinque-radiate symmetry in the ontogeny of its skeletal plates.

The spines arise as calcifications in the superficial layer of the dermis, and are at first numerous and uniformly distributed over the abactinal surface, except that they appear latest in the region of the last-formed rays. We next find them set singly, or in groups of twos or threes—the first paxillæ—over the larger skeletal plates.

The development of the ambulacral and adambulacral plates follows the course described by Ludwig in Asterina (**12**) and by the author in Solaster endeca, the first ambulacrals being proximal to the first tube-feet in each ray, and the first adambulacrals forming the mouth-angle plates. However, from careful maceration experiments, it appears that each first ambulacral is continuous in origin and early growth with the corresponding first adambulacral or mouth-angle plate, the joint which finally separates them being of secondary formation. This is interesting in view of the fact that palæozoic Asterozoa (Spencer, **24**, p. 30), have no ambulacral plates proximal to the first tube-feet. We may infer that the first ambulacrals of recent starfish are new formations, and that they have arisen as buttress extensions from mouth-angle plates towards the middle of each ray. This will throw light on the distinction between the adambulacral and the ambulacral types of mouth-armature in recent starfishes, the former being presumably the more primitive.

In any case, two important facts have been established regarding particular "primary" plates: (1) That in the terminals we have an example of fusion of previously-formed calcifications, and (2) that in the first ambulacrals the converse process, namely, separation from a larger calcification, is exemplified.

General.

Among features of general interest in the foregoing which claim further notice are the following: (a) The mode of origin of the cœloms; (b) the mode of formation of the dorsal sac or madreporic vesicle; (c) the origin of the pharyngeal or perioral cœlom; (d) the origin of pocket I II of the external oral perihæmal ring; (e) the sequence in the formation of the hydrocœle pouches; (f) the relation of the larval stalk and sucker to the hydrocœle ring; (g) peculiarities in the development of the skeleton (see above).

(a) Origin of Cœloms.—Neither in Solaster endeca (**7**) nor in Crossaster papposus is there any indication of an origin of the posterior cœlom by constriction from the anterior cœlom, and the gut is an isthmus, not a diverticulum of the archenteron. In Asterias rubens (**8**), A. glacialis (**10**) and Porania pulvillus (**9**) I described rudiments of an independent posterior cœlomic outgrowth from the enteron. In Enteropneusts, Morgan (**20**, p. 413) and Bateson (**1**) have ascribed a posterior origin to the trunk cœloms, while Davis states that these arise through backward growth of the anterior cœlomic sacs. In Asterias rubens (**7**), as in Asterina (**12**), the hypogastric cœlom of the adult is derived entirely from the larval left posterior cœlom. I have accordingly put forward (**8**, p. 234) the view that in Enteropneusts and Echinoderms generally there are two potential modes of origin of the posterior cœloms: (1) by constriction from an anterior cœlomic outgrowth, and (2) by a separate posterior outgrowth from the enteron. As regards Crossaster and Solaster, the conclusion is drawn that both modes of origin are represented, the former on the right side of the larva and the latter on the left side. Thus on the right side the epigastric cœlom takes origin as a backward diverticulum of the anterior cœlom, while on the left side the hypogastric cœlom arises independently behind the enteron. In this connection it will be remembered that actually at any early stage the right lateral diverticulum of the anterior cœlom extends further back than the left diverticulum (Text-fig. 4), while the posterior cœlom has frequently its greatest extension dorsally, and to the left. (See also discussion in **8**, p. 234.)

(b) Dorsal Sac.—It was a great surprise to find that in Crossaster and Solaster the dorsal sac takes origin from the epiderm. I hesitated to publish this result until I had obtained and examined sets of ova from three separate spawnings. MacBride and Masterman state that the sac in question has a cœlomic origin in Asterina (**13**) and Cribrella (**19**) respectively. I had recently the opportunity (1916) of verifying this statement beyond the possibility of doubting in the case of Cribrella from reared larvæ. As regards Solaster endeca, when I worked out its development (1910) the evidence was not at first conclusive, but later some of my material showed the sac arising from the anterior cœlom, and accordingly I stated (**7**, p. 26, 1912) that, so far as I could make out, the dorsal sac is budded off from the anterior cœlom. But unfortunately this later material had been supplemented by plankton-gathered larvæ (**19**), and along with these a few Cribrella larvæ prove to have been collected, and to have been mistaken, most regrettedly, for Solaster. At that time it had not been put on record, as I have since done (**10**), that Cribrella eggs may be pelagic, a brooding habit being stated to be characteristic of the species (**19**). The two species have the same spawning period, and their eggs and larvæ resemble one another during early stages, and although the anterior brachium normally remains single in Solaster and becomes double in Cribrella, still it is occasionally doubled in the former.

I cannot suggest any satisfactory explanation which will reconcile the epidermal origin of the sac in Crossaster and its cœlomic origin in Asterina and Cribrella, etc., and its apparently mesenchymatous origin in certain other forms. Possibly the re-arrangement of the central cells during blastula formation may provide opportunity for portions of tissue from deeper layers being lined up thus early with the epiblast. Or, again, in larvæ like that of Echinoderms, it is by no means inherently out of the question that the formative tissue of a muscular sac destined to lie close under the skin should actually arise from epiderm. But the want of uniformity is the puzzle, and meantime, apart from Crossaster and Solaster, the position is that in Asterina (**13**), Cribrella (**19**), Echinus (**15**), Ophiothrix (**14**) and Echinocardium (**17**) a cœlomic origin is assigned to the sac, while in Porania (**9**), Asterias rubens (**8**) and A. vulgaris (**4a**) the sac is said to be of mesenchymatous origin, and to be developed from a cell or cells appearing close under the epiblast. In Enteropneusts, Bateson (**1a**) and

Morgan (**20**) speak for a mesenchymatous, and Spengel (**25**) and Bourne (**2**) for an epidermal origin of the pericardial sac with which the madreporic vesicle of Echinoderms is no doubt homologous. To suggest that in the Solasters the madreporic vesicle corresponds to an enteropneust neural cavity which has somehow replaced a closely adjacent pericardium of enterocœlic or mesenchymatous origin is a speculation hardly stranger than the actual ontogenetic facts.

As a matter of fact, in Crossaster papposus and Solaster endeca the pit of origin of the madreporic vesicle is continued ventrally along a distinct ectodermal groove, from the bottom of which ectodermal cells are budded off towards the wall of the future epigastric cœlom. I have not yet been able to ascertain the fate of these cells, but certainly they do not originate in the epigastric cœlomic wall. Perhaps they are the fundament of the aboral nervous system, and correspond with the posterior dorsal nodes described by me in the larva of Porania (**9**, p. 42).

(c) Origin of Pharyngeal or Perioral Cœlom.—Crossaster agrees with Cribrella and Solaster in having this cœlom arising as a series of pockets from the posterior cœlom, one in each interradius. On the other hand, in Asterias rubens (**8**), Asterina (**13**) and Asterias pallida (**11**) the pharyngeal cœlom arises by a single outgrowth in the neighbourhood of interradius I/II. The fact that in Crossaster the pharyngeal outgrowth in interradius I/II does not lose its connection with the posterior cœlom goes some little distance on the way to reconcile the above divergences. We may see in these facts an indication borne out by other data that interradius I/II is to be counted as the first or fundamental interradius in Asteroid symmetry. In Crossaster the interradial origin of the pharyngeal cœlom is rendered in some respects more difficult, but in other respects easier to demonstrate by the fact that the development of the series of pockets is spread over a relatively long period.

(d) Origin of External Oral Perihæmal Ring.—Crossaster agrees with Solaster (**7**), Asterias rubens (**8**) and Cribrella (**10**) in having all the pockets for this sinus arising from the posterior cœlom. On the other hand, in Asterina (**13**), Ophiothrix (**14**) and Cribrella (**19**) perihæmal pocket I/II is described as arising from the axial sinus portion of the anterior cœlom. In all probability the apparent discrepancy is related to the fact that a communication is established between the anterior cœlom and the dorsal horn of the posterior shortly before the formation of the perihæmal pocket in question. Indeed, in Cribrella (**10**) I have demonstrated the posterior cœlomic origin of this pocket. It seems in every way probable that primitively the whole of the perihæmal pockets arise from the left posterior cœlom. In Echinus all five pockets for the lantern cavity—a cavity which MacBride holds to be homologous with the external oral perihæmal sinus—take origin from the left posterior cœlom (**15**), while the corresponding pockets in Echinocardium have a similar origin (**17**).

(e) The Sequence in the Formation of the Hydrocœle Pouches.—In this connection the most striking feature is that while pouches I to V arise practically at the same time and VI very slightly afterwards sinistral to V, the remaining pouches arise partly in series sinistral[1] to V and partly in series dextral[1] to I. In Solaster endeca the pouches additional to the five primary ones either all arise in series sinistral to V (eight-rayed specimens), or the last of them arises (nine-rayed specimens) dextral to I from the dorsal horn of the hydrocœle crescent (**10**). We can say that there is a tendency towards secondary bilateral symmetry in the development of the later hydrocœle pouches of Crossaster (see Pl. 10, figs. 7 and 8). At the same time the two great landmarks of starfish radial asymmetry, namely, the madreporic complex and the anus in interradii I/II and V/VI respectively, show that, in Crossaster, rays VI to XII, like VI to IX in Solaster, are to be regarded as a secondary addition, and that the other rays are rightly numbered by counting the madreporic interradius as I/II, with I on the dextral side of the madreporic opening.

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[1] See note on p. 156.

Relation of Stalk and Sucker to Hydrocœle Ring.—It is significant to note (p. 161) that in Crossaster and Solaster the last remains of the sucker are external to the hydrocœle ring, which never actually encircles the larval stalk, as it does in Asterina (**13**) and Asterias (**8**). The difference is probably due to the late period at which the hydrocœle ring is completed in Crossaster, though this reason cannot apply in the case of Cribrella, where the ring in question is closed at an early stage without enclosing stalk or sucker. There is, however, every ground for thinking that Echinoderm larvæ with a large open hydrocœle crescent (Asterina, Asterias, Crossaster, Solaster) are more primitive in this respect than those in which the hydrocœle ring is closed practically from the first.

SUMMARY AND CHRONOLOGY OF DEVELOPMENT.

Segmentation total, equal or subequal (1 to 2 days). Formation of hollow blastula by egression (3 to 4 days). Formation of gastrula by invagination (5 to 6 days). Elongation of gastrula and division of archenteron into anterior, middle and posterior larval cavities (7 to 8 days). (In course of later development the anterior larval cavity gives rise to cavity of præoral lobe, hydroporic canal, hydrocœle, epigastric cœlom, axial sinus, stone-canal, axial organ, internal oral perihæmal ring, and oral hæmal ring. The middle larval cavity gives rise to all portions of the adult digestive cavity. There is no larval mouth, œsophagus or anus. The posterior larval cavity gives rise to the hypogastric cœlom, the pharyngeal or perioral cœlom, the external oral perihæmal ring, the "genital rachis" and the aboral perihæmal sinus.) Indication of brachia and sucker (9 to 10 days). Formation of hydroporic opening (11 to 12 days). Closure of blastopore; formation of dorsal sac or madreporic vesicle from epiblast (12 to 14 days). Hydrocœle pouches I–VI appear (15 to 17 days). First indication of developing aboral and ambulacral skeletal plates (17 to 18 days). Flexion and torsion of præoral lobe begins (18 to 19 days). Ultimately the preoral lobe is absorbed or incorporated with the oral (larval left) side. Sucker fixation (19 to 20 days). The hydrocœle lobes appear externally in the following sequence: II–IV (21 to 22 days), I (22 to 23 days), VII (27 to 28 days), XII (29 to 30 days), VIII (33 to 34 days), IX and XI (37 to 41 days), X (43 days). First pairs of sucker feet on rays I–VI begin to be functional and the larvæ shift position slightly while still anchored by the sucker (28 to 30 days). Sucker fixation lost (35 days). Mouth opening (38 to 42 days) and anal opening (49 to 55 days) appear. The oral and aboral gastric ligaments arise by partial breaking down of the mesenteric sheets separating the perioral and hypogastric cœloms from the hypogastric and epigastric cœloms respectively.

I have to express indebtedness to the Carnegie Trust for grants towards meeting the expenses of this investigation, and to Mr. John Peden, Laboratory Attendant at the Millport Biological Station, for skilful and ungrudging help in obtaining and rearing the eggs and larvæ required, in the absence on active service of Mr. R. Elmhirst, Superintendent of the Station.

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EXPLANATION OF PLATE 10,

Illustrating Dr. James F. Gemmill's paper on "The Development of the Starfish Crossaster papposus, Müller and Troschel."

My paper on the development of Solaster endeca (7) may be referred to for figures which illustrate the following features in the development of Crossaster papposus: Segmentation, blastula and gastrula formation, Pl. i, figs. 1–6; separation of primary cavities in the larva, Pl. iii, figs. 24–33; general growth and mode of attachment of the larva, Pl. ii, figs. 15–21; sections of larvæ and of stages in metamorphosis, Pl. iv, figs. 36–48; various points in the development of the skeleton, Pl. i, figs. 8 and 9, and Pl. ii, fig. 30.

Abbreviations employed in Reference Letters both in Plate and in Text-figures.

a.a. Anterior brachium. *a.c.* Anterior larval cœlomic cavity. *amp.* "Ampulla of stone-canal" (see p. 172). *ar.* Aboral arm-rudiment. *ax.o.* Axial organ. *ax.s.* Axial sinus. *ax.p.* Main opening from axial sinus into pore-canal system. *bl.* Blastopore. *br.* One or other of the lateral brachia. *cbr.* Cavity of præoral cœlom running up into a brachium. *cld.* Left lateral diverticulum of anterior larval cœlomic cavity. *d.h.* Dorsal horn of posterior cœlomic cavity. *d.s.* Dorsal sac or madreporic vesicle. *ect.* Ectoderm. *ep.c.* Epigastric cœlom (right lateral diverticulum of anterior larval cœlomic cavity. *g.* Enteron. *g.h.t.* Gastric hæmal tufts. *hy.* Hydrocœle. *hyd.* Hydroporic canal or opening. *hyp.c.* Part of hypogastric cœlomic cavity. *m.a.p.* Cushion over mouth-angle plates carrying spines. *mth.* Mouth. *mes.*

Mesoblast filling space between the two primary embryonic layers. *nb.* Aboral brachiolarian notch (in interradius I/II). *p.c.* Posterior larval cœlomic cavity (future hypogastric cœlom and other derivatives). *ph.* Pocket from posterior larval cœlomic cavity for one of the segments of the external oral perihæmal ring. *pr.c.* Præoral cœlom. *pr.l.* Præoral lobe. *s.* Sucker of larva for attachment during metamorphosis. *s.f.* Sucker-foot of young starfish. *sp.* Papillæ containing developing spines. *st.c.* Stone-canal. *v.h.* Ventral horn of posterior larval cœlomic cavity. *v.w.* Communication of "heart" with spaces in aboral body-wall.

PLATE 10.

Figs. 1–5.—Late free-swimming larvæ of Crossaster, showing the relations of the more important larval cœlomic cavities. The outline of the gut-wall is dotted in these figures.

Fig. 1.—Fifteen days from larval left side.

Fig. 2.—Fifteen days from larval dorsal side. (Dorsal sac and hydroporic canal are not shown in this figure.)

Fig. 3.—Fifteen days from larval ventral side.

Fig. 4.—Fifteen days from larval right side.

Fig. 5.—Seventeen days with six fully developed hydrocœle pouches and a commencing seventh. The præoral lobe is beginning to be flexed and twisted towards the larval left side.

Fig. 6.—Stage 21 days old (2 to 3 days after attachment) from oral (left larval) side. The first six hydrocœle pouches now show three or five lobes (the future podia) while the seventh pouch is still simple. The præoral lobe is well flexed back on the left (larval) side, but has not yet begun to be absorbed.

Fig. 7.—Stage 28 days old (8 days after fixation), showing gradual reduction of the præoral lobe, increase in the future circumoral area, further development of the sucker-feet on hydrocœle pockets I–VI, and commencing formation of pouch XII from what was the larval dorsal horn of the hydrocœle crescent. The hydrocœle ring is incomplete in the region of the sucker.

Fig. 8.—Stage 37 days old (16 days after fixation). The præoral lobe is now absorbed all but the sucker. Hydrocœle pouches VIII and XI are definite, while IX and X are just apparent, and the hydrocœle ring-canal is in process of completion between them, the sucker being outside the hydrocœle ring. The tube-feet on rays I, VII and XII are functional and the little starfish shifts position, though it is still anchored by the sucker, which has not yet lost its hold.

Fig. 9.—Young Crossaster with all its rays developed and the sucker almost completely lost (20 days after commencement of fixation and 3 days after detachment of sucker). Note the relative backwardness of differentiation on the part of the rays in the segment between pouches XI and VII. Note further in figs. 6–8 a semblance of bilateral symmetry produced by the mode of development of the later-formed rays on either side of the sucker.

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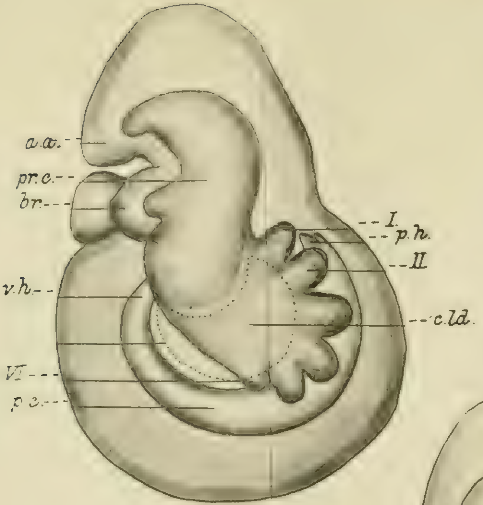

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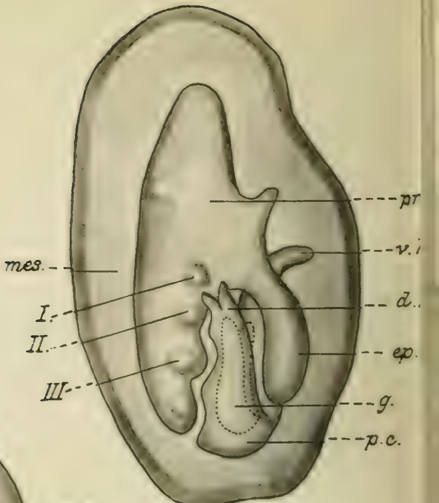

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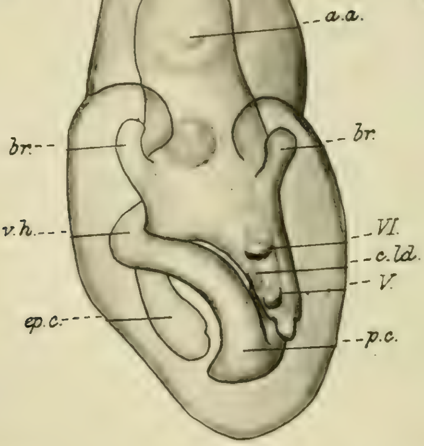

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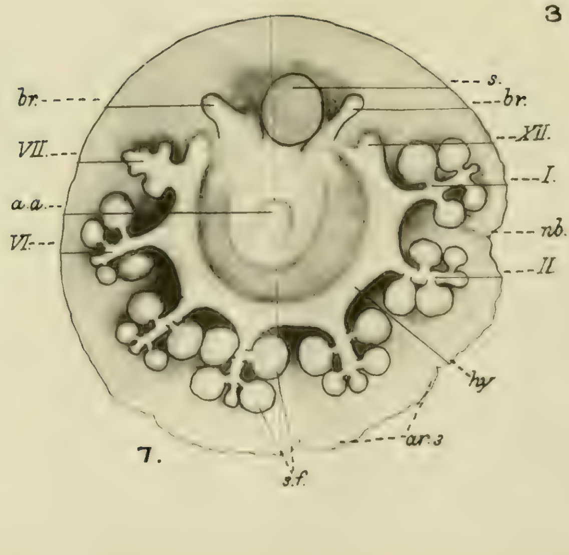

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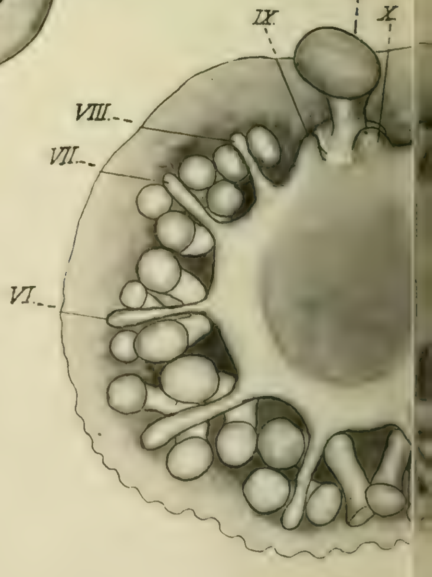

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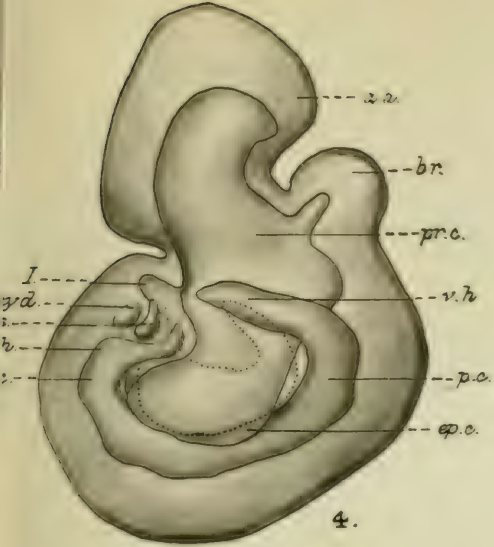

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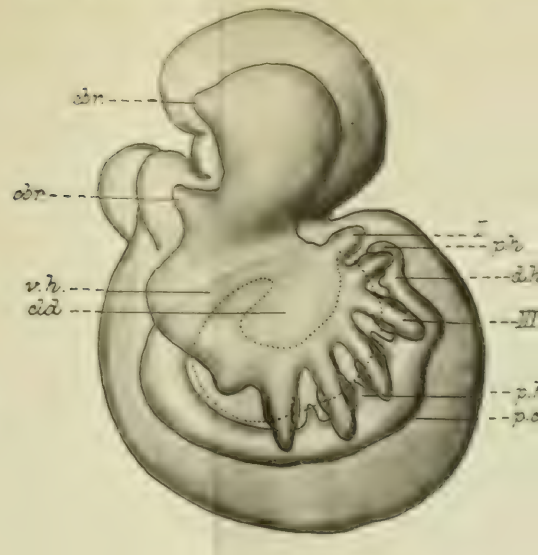

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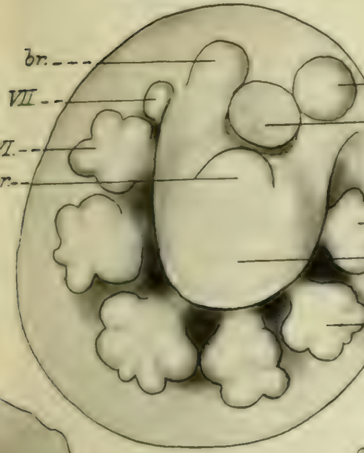

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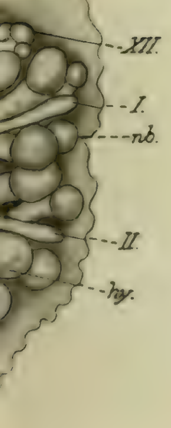

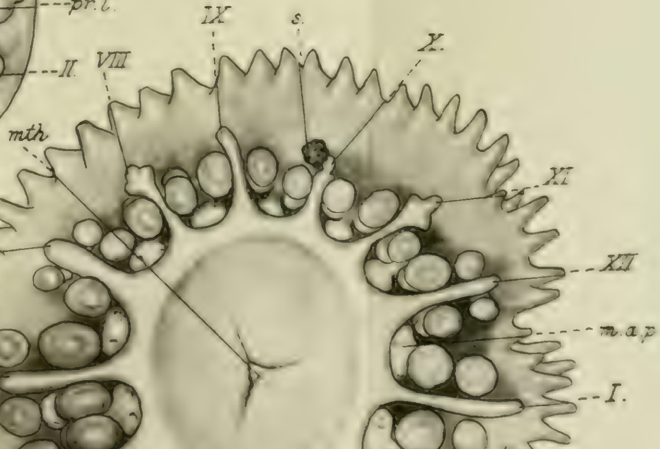

9.

CROSSASTER.

Huth, London

Determination of Age in Honey-Bees.

By

Helen L. M. Pixell-Goodrich, D.Sc.

With Plate 11.

It is of the utmost importance in the study of certain bee diseases to be able to separate definitely bees dying of a specific disease from those which are merely dying of old age. In the height of the honey-flow worker bees are said literally to work themselves to death in about six weeks after hatching. Consequently during the summer the normal daily mortality is very high—from a hive of fifty thousand workers being at least several hundreds. The last bees hatched out in autumn hibernate with the queen and thus attain an age of several months. It is popularly supposed and stated in some books on apiculture that an old bee may be recognised by her worn appearance, her body tending to be hairless and her wings frayed. The inaccuracy of this general statement and the uselessness of such a diagnosis for scientific purposes was soon apparent. I have carefully examined bees of many months in age (e.g. autumn bees flying in May) and compared them with those of a few weeks without finding any constant external differences. Conversely, relatively young bees may become hairless and their wings frayed under exceptional circumstances.

As a rule old bees die away from home, possibly during a foraging expedition, but they sometimes appear to return, especially to a weak colony, and have ultimately to be turned out of the hive by its younger and more vigorous members. Bees having so few ways of showing their symptoms, it will be readily understood that there is much difficulty in differentiating disease from senescence at sight. If a bee is ill or very old it will first be unable to fly and later hardly able to crawl. Many "bee-experts" diagnose a crawling bee to be suffering from "Isle of Wight"[1] disease if its rectum is distended with an accumulation of undigested pollen, wax, etc., which can be readily squeezed out by slight pressure. It must be remembered, however, that, since bees normally defæcate on the wing, there will tend to be a collection of such undigested matter in any bees unable to fly.

Before proceeding further it will be well to explain briefly how far the investigation of the common diseases of adult bees has progressed in this country. Among diseases which can be diagnosed microscopically or bacteriologically are:

(1) Microsporidiosis or Nosema disease due to the protozoan parasite Nosema apis, Zander, which has been described by Fantham and Porter (**7**). Much has been written on this disease (**7a**, **20**), which is not at present at all common in England. No doubt the activities of the Board of Agriculture since 1906 have helped to reduce the frequency of its occurrence.

(2) A form of dysentery or inability to retain fæces appears to be due to a small oval yeast—possibly of more than one species. The contents of the colon in affected bees are often watery, and always show enormous numbers of actively budding yeasts which are also present in the ventriculus. These organisms, which stain readily by the Gram or Claudius method, have never been found in the walls of the alimentary canal nor in other tissues. They are readily stained and therefore presumably killed by methylene blue, so that no doubt this and other mild antiseptics help to reduce their numbers. In these disorders, therefore, spraying and medicated feeding are likely to have some beneficial effect.

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[1] See footnote to p. 193.

It must be remembered that yeasts are very widely distributed organisms, so that hardly a bee examined will be free from small numbers taken in with pollen grains, etc. They are usually, of course, quite harmless, and it is only when conditions are suitable for them to pullulate that they appear to have any pathogenic effect.

(3) After a bee has been free in a room for even a short time I have found that it almost invariably develops one or more colonies of a mould. Branches of the mycelia of these often penetrate the walls of the alimentary canal, causing adhesions between its various parts and rapidly proving fatal. The spores of these fungi are no doubt ingested by the bee with dust from the room.

(4) The black shiny condition occasionally met with in some bees of a stock owing to loss of the normal covering of hairs has been associated by Cheshire (**2**, ii, p. 570) with the presence of an organism—Bacillus gaytoni—in the intestine. This finding has not so far been confirmed. Cheshire states that in such cases the queen is generally badly affected and that re-queening is the best way of checking the disease.

In addition to these there would seem to be at least one other disease for which a specific organism has not yet been separated. This I shall refer to provisionally as malignant disease.[1] Rennie (**17**) has recently published some interesting experiments on the infectiousness of this disease. On no occasion have I been able to obtain any bacteria from the blood, muscles or fat body of bees examined bacteriologically. From the alimentary canal so vast is the number of bacterial colonies usually obtained with ordinary nutrient media that the difficulty is to separate them for identification. No organisms have so far been demonstrated actually inside the cells of the alimentary canal although I have stained numerous serial sections with almost every conceivable stain, including Gram, Van Gieson, Claudius, etc. As might be expected, the intestines of bees from almost every different locality contain different bacteria which will grow on various media, some aërobically, others anaërobically. So long as I was uncertain whether a crawling bee was merely senescent or really diseased, the problem of finding whether any special bacterium was associated with the disease was practically impossible to solve.

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[1] It is better to avoid the use of the term "Isle of Wight" disease owing to the confusion associated with it. This mysterious unknown disease (malignant disease) has been much confused with microsporidiosis. Further, one or two instances in which yeasts have been mistaken for Nosema by incompetent observers posing as experts have come under my notice. Thus at times at least three of the above disorders have been called "Isle of Wight" disease, and it is now, I fear, impossible to determine with certainty to which the term was originally applied.

Much has been written also about bee paralysis, but I can see no reason for considering this to be a distinct disease. Supposed isolated instances are doubtless often due to accident—the result of fighting amongst themselves or with wasps, etc.—age and cold, or, as Malden (**7a**, p. 135) points out, many so-called cases are in reality cases of the above (malignant disease).

The possibility of determining the age of bees is likely to be of further importance in estimating the chances of survival of a stock during the winter. There is no doubt that age and suitable general conditions of life have much to do with successful wintering, and that loss of stock owing to lack of these conditions is often quite wrongly attributed to disease.

Being then convinced of the necessity of being able to determine the age of a bee. I carefully examined, in the first place, such exterior parts of the body as the hairs, glossa, gonapophyses, etc. On concluding that nothing definite was to be determined from these, the study of the brain and head glands was begun.

The work of Minot (**15**) and others, together with the fact that a bee appears to work incessantly during its short life, suggested that the nerve-cells would give a good indication of age. The results so far obtained show that a great deal may be learnt from a study of the brain and glands of the head.

Normal Life-history of a Worker Bee in Summer.

After laying of fertilised egg	1st–3rd day	Incubation of egg.
	3rd–8th day	Larva is hatched and fed by workers (nurse bees).
	9th day	Cell containing larva is sealed with a capping of wax and pollen.
	9th–22nd day	Larva spins a cocoon and becomes a pupa or chrysalis, then a perfect insect (imago).
	22nd day	Bites her way through capping.
After emergence from cell	For 1st two weeks	Young bees remain in hive acting as nurses, etc.
	From 2nd to ? 6th week	Fly backwards and forwards to hive on foraging expeditions.

For the experimental work a revolving observatory hive was fixed on the laboratory bench with its passage for exit communicating with the outside air by a small hole in the mullion of a window. The hive itself held only two combs taken with brood and a queen from an ordinary hive. The observatory hive was connected above with a glass-fronted "super" containing four sections and room for more combs. The bees were fed, when necessary, through holes in the top of this "super." One of the ordinary glass sides of the hive was replaced by a specially designed one, consisting of four glass doors in narrow frames, each opening independently and commanding one-half of a comb. By means of these doors bees at required stages could be removed without disturbing the whole hive. A record kept of the date of sealing of some of the larvæ enabled me to remove imagines on the eve of hatching. Other bees were taken out with padded forceps as they bit their way through the cappings of their cells, and either used for experiment or marked and put back.

Marking proved to be a decided difficulty. A dab of white or light-coloured oil-paint on the thorax would sometimes remain visible for two or three weeks if it dried quickly enough and was of the correct consistency. Pigment mixed with gum-arabic was no good at all, for on drying it came off in a lump with any hairs of the thorax that it touched.[1] Attempts were made to tinge the wings by applying a dye dissolved in water, alcohol, chloroform or ether, but no penetration could be effected.

The least unsatisfactory method of marking in some ways is to clip the tip of one or more wings. This does not interfere with flight so long as only the very tip is removed. By clipping different combinations of the four wings any number at a time (2^4-1), i. e. fifteen different dates of hatching could be indicated. The drawback to this method is, of course, the difficulty in recognising a clipped bee except at very close quarters.

For keeping bees under special observation I use large glass bell-jars in which they can take short flights, and will sometimes live for three or four weeks. Each jar is provided with a large piece of crumpled paper in which the bees can hide from the light, and honey, syrup or candy and pollen are supplied at intervals. By adding various drugs to the food it is easy to test their action, but on the whole wasps or bumble-bees are better for feeding experiments since they live longer in captivity.

TECHNIQUE.

After some preliminary experiments the brains were found to be best fixed for the routine study of nerve-cells in the following way: The whole head is cut from a living bee, after subduing with chloroform, if necessary. The upper part of the chitin (just above the three simple eyes) is quickly sliced off and the head placed in a small tube of Petrunkewitsch fixing-fluid for about fourteen hours. After thoroughly washing in 70 per cent. alcohol containing iodine, the head is transferred to 90 per cent. alcohol for some hours. It is then easy to remove the remainder of the chitin from the posterior part of the head, and to lift out the entire brain (Pl. 11, figs. 1 and 2) with the subœsophageal ganglion and some of the head-glands attached. After further washing the brain is prepared for embedding and cut into serial sections as required.

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[1] Since writing this I have heard from Mr. Bullamore that at the Cambridge Institute of Bee-keeping they have added to the pigment used some of the preparation known as "new skin," and in this way managed to distinguish bees for three or four weeks. Certainly the addition of the celloidin solution is an improvement, appearing to give elasticity and so prevent the pigment from coming off so quickly. However, even this has a tendency to peel off and bring the thoracic hairs with it, so that I am afraid it may still be difficult to recognise bees after four or more weeks, and these are the ages at which specimens are now required for study.

Sagittal sections were cut of some brains, but generally transverse sections are more useful. These are cut from above downwards, starting through the three simple eyes. By leaving the optical pigment attached to the brain it is easy to orientate it when embedded.

For routine examination of individual bees transverse sections 7 μ thick are preferred, and they are stained with Ehrlich's hæmatoxylin and orange G. Many other stains were tried. Among them carbolthionin was good for brain-cells, and alcoholic carmine with picronigrosin as a counter-stain was useful for picking out membranes and connective tissue. Perfect fixation of the material immediately on death is, of course, most important in order to exclude error arising from post-mortem changes.

Ohlmacher's fixative penetrated very well, but owing to the large percentage of absolute alcohol (80) and chloroform (15) present, all the lipoids of the cells were dissolved, producing intense vacuolation throughout. Fleming's solutions, on the other hand, were not sufficiently penetrating for the whole brain. No doubt by employing some of the osmic acid mixtures without acetic acid on small portions of the brain or other ganglia and staining by the more elaborate methods, much more interesting detail might be made out in the cytoplasm. I have not, however, myself felt justified in spending time on these methods, since all that is required for the pathological work undertaken for the Board of Agriculture is to discover whether a crawling bee sent for examination is senescent or not. If the nerve-cells are found to be in sufficiently good condition to rule out the possibility of senescence, it is then worth while to spend some weeks on the complete investigation of the bacteria of the bee, on the chance of finding organisms which are possibly pathogenic.

Attempts were made to do away with the necessity of fixation at all and to examine the nerve-cells in a fresh state. For this purpose the abdominal as well as the cerebral ganglia were investigated. Injection with methylene-blue has not proved successful with bees (I cannot find any instances in which it has succeeded with adult insects). It must be remembered that the ganglia are surrounded with a considerable thickness of connective-tissue, and in addition a close net-work of tracheæ makes their opacity very great.

The abdominal ganglia were sometimes rapidly removed from a bee opened under Ringer's solution (Locke's modification) as usual and then flooded with a dilute solution of methylene-blue in the same solution (·001 per cent.), but penetration was always difficult, and the cells could only be made clear after a considerable amount of teasing of the ganglionic tissue. Consequently these processes had to be abandoned in favour of a constant method of fixation as described above.

Experimental Results.

In the serial transverse sections cells of several different sizes are to be found (**11, 18**). The cells that I have studied are the large ones with a considerable amount of cytoplasm when young, and a nucleus measuring 8 by 10 microns or more. No definite Nissl bodies are to be seen after the fixation used, but some of the older cells are hyperchromatic, having a varying number of irregular masses of deeply-staining substance in the cytoplasm (Pl. 11, fig. 8). These large cells are found chiefly in the following four main regions:

(1) Almost at the centre of the brain is a wedge-shaped mass of cells pointing towards the anterior surface, and

having as a base a group of giant fibres. In each of the dozen or so sections, 7 μ thick, passing through this region there are generally four or five large cells—rather more in very young brains.

(2) Cells of the antennal lobes. Each of these lobes consists of a convoluted spherical mass of fibres giving rise to the antennal nerve and surrounded by nerve-cells. These are the cells studied by Hodge (**9**, **10**) and Smallwood and Phillips (**18**).

(3) Just anterior to the inner fibrillar body of the optic lobe is a mass of cells, chiefly small ones, but with a few giant ones among them.

(4) Cells of the subœsophageal ganglion. The mass of fibres forming the centre of the ganglion and giving rise to the ventral nerve-cord is surrounded by a layer of cells which is especially thick on its lower surface. It has been usual to consider the subœsophageal ganglion as part of an insect's brain (**16**, **8**), since Faivre's physiological experiments (**6**) appeared to show that the power of co-ordinating the movements of the body is there situated. This ganglion therefore appears to correspond to the cerebellum of vertebrates.

The large cells, with nuclei 8–12 μ in diameter, from these four regions in one and the same brain show great constancy in appearance. They are also very similar to those from other bees of the same age, but exhibit constant differences from those of bees at other ages.

On the whole it is most convenient to study the cells from the fourth region, i. e. the subœsophageal ganglion. The large cells here are very numerous, forming several layers below the mass of fibres going to the ventral nerve-cord, and bounded on each side by small groups of fibres, from which the mandibular, maxillary and labial nerves arise (Pl. 11, figs. 3 and 4). These layers of cells appear in about ten serial sections, and are therefore about 70 microns thick. In young bees they consist chiefly of large plump cells, only separated from each other by narrow strands of connective tissue with occasional connective-tissue cells (Pl. 11, fig. 3). As the bee increases in age, vacuoles appear at the periphery of the cytoplasm of the nerve-cells, so that even at the end of the first week of toil most of the cells have somewhat the appearance shown in Pl. 11, fig. 6. During the exertions of foraging for nectar, pollen, etc., the brain-cells age still further; so that a bee returning to the hive at mid-day laden with pollen showed the condition represented in Pl. 11, fig. 7.

The cell shown in Pl. 11, fig. 8, came from the sub-œsophageal ganglion of a bee which was unable to fly, and had lost nearly all the hairs on its thorax. There is thus good reason to think that it was senescent. The final stage of disintegration of the cytoplasm is shown in Pl. 11, fig. 9, one of the cells from the ganglion drawn in Pl. 11, fig. 4. This belonged to a bee which came from a hive on a fine day in March, but was too weak to effect a cleansing flight and soon became moribund. It will be seen that the nerve-cells are quite worn out, there being in this part of the ganglion only a framework of connective tissues, with here and there the nucleus of a nerve-cell in a more or less necrotic condition, with only a trace of cytoplasm round it.

It will be noticed that the nuclei of bee nerve-cells contain several nucleoli as described by Binet (**1**) for most insects. Dolley (**3**), however, figures for the crayfish a single large nucleolus such as is found in typical vertebrates. I cannot agree with Mann (**13**), Hodge (**9**), and some others that there is a marked shrinkage of the nucleus due to fatigue. In well-fixed brains the nuclei appear to retain their normal shape and also their size, as shown by Smallwood and Phillips (**18**). In very old bees, however, the chromatin seems generally to leave the nuclear membrane and tends to form a single clump just inside.

A reduction of cytoplasm due to fatigue has been previously described by Dolley (**3**) in the nerve-cells of the crayfish after artificial stimulation. This he refers to as an œdematous condition, since the so-called vacuoles left at the periphery of the cell as the protoplasm disintegrates become, of course, filled with fluid. In crayfish and other animals which live for some years with intervening periods of rest one would expect to find alternating signs of fatigue and recuperation in the cells such as he describes. In bees, as far as can be ascertained, there is no nocturnal rest. The specimens for experiment were, however, taken from the hive about noon. In this way it was hoped to eliminate any differences which might possibly arise from daily fatigue. It seems very doubtful, however, in spite of Hodge's results (**9**), whether there is any recuperation during the night. Except that bees do not fly after dusk, the various activities appear to go on as usual. Certainly wax secretion and building of comb, transference of honey or sugar from one part of the hive to another, ventilating, guarding, etc., are carried on throughout the night. It would be most surprising to obtain constant differences in the nerve-cells of bees caught on the wing in the morning and evening without taking their age into account as first attempted by Hodge.

Dolley (**4**) has shown that incessant exercise has a very decided effect on the nerve-cells of artificially stimulated dogs as well as of the crayfish as described above. One is therefore not surprised to find that the incessant work done by a bee produces a pronounced cumulative effect, and further, since the bees normally work themselves to death in so few weeks, that the condition of the nerve-cell gives a good indication of age in bees.

During successful hibernation no doubt a certain amount of repair takes place in the brain and other tissues of the bee. I hope to study these recuperation stages in more detail during the winter.

Some bees which appear to be dying of disease have been found to have nerve-cells rather less aged than those of the active forager shown in Pl. 11, fig. 7. It seems likely that loss of activity consequent on attack by disease would prevent, if anything, the usual disintegration of the nerve-cells, so that a diseased bee would appear, if studied from this point of view, to be younger than it really is.

If these determinations of age are to be made of general use, it is important to eliminate as far as possible the personal equation. This can be done to some extent by comparing the number of giant nerve-cells in some constant region of the brain. There appears to be a small decrease in the number of these cells with age, as stated by Hodge (**10**), and as has been shown also for the Purkinjë cells of the human cerebellum by Ellis (**5**). However, the counting of them entails much labour, and consequently the tables with these numbers will be held over until next year, when I hope to have a perfect series of brains at all ages.

Further, the condition of the glands gives some clue to age, for in normal healthy bees their physiological condition corresponds to actual age.

The glands of the head that are being studied are the pharyngeal, salivary, mandibular and maxillary as described by Snodgrass (**19**) and Cheshire (**2**). In addition, a small pair of glands has been discovered of which I can find no previous mention (Pl. 11, fig. 2). These occur one on each side of the œsophagus as it emerges from between the supra- and subœsophageal ganglia—that is, they are just in front of the commissures. These œsophageal glands are almost spherical, about 120 microns in diameter. From each proceeds what appears to be the remains of a convoluted duct to meet its fellow of the opposite side at an angle of 60° above the œosphagus and in close contact with the central portion of the supra-œsophageal ganglion.

For the full investigation of these various head glands much study is still necessary, and experiments with intra-vitam stains are being carried on. Some of the glands vary enormously with the immediate function of the bee. The pharyngeal glands, for example, become distended with secretion soon after hatching and remain so during the two weeks that the bee functions as a nurse. By the time that pollen gathering is undertaken, these glands appear to be exhausted and in an advanced hyperchromatic state which gives the collapsed glands a necrotic appearance. The different histological appearances at the various ages promise to give most interesting results as to the cause and effects of disease, as well as the elucidation of many points in the life-history of bees, such as the process of elaboration of the varying bee foods—royal jelly, etc.,—and the ages at which these activities are carried on.

It is a pleasure here to express thanks to Prof. G. C. Bourne, in whose department this work is being carried on, and to my husband, Mr. E. S. Goodrich, for their interest and valuable advice during the progress of the work. Mr. J. B. Gatenby has also made many helpful suggestions in connection with the treatment of insects for cytological examination. I also wish to record that this work on bees is being carried on by means of a grant from the Development Commissioners of the Board of Agriculture. Much assistance in obtaining bees suspected of disease has been given by Mr. A. G. L. Rogers, and more recently by Dr. Keeble and his assistants in the Horticultural Section of the Food Production Department.

SUMMARY.

For the study of those bee diseases with which no specific organisms have so far been identified, it is important to be able to eliminate bees dying of old age, and this cannot be done with certainty by observing outward symptoms. However, the age of bees, which normally work almost incessantly for about six weeks and then die, may be determined with some accuracy from a study of the brain-cells. With advancing age the cytoplasm of these cells undergoes gradual reduction peripherally, until in senescence only a vestige is left surrounding the nucleus.

The condition of the head-glands, including a pair of œsophageal glands which do not appear to have been previously recorded, gives some indication of age in normal healthy bees.

UNIVERSITY MUSEUM,
OXFORD;
September, 1919.

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EXPLANATION OF PLATE 11,

Illustrating Mrs. Helen L. M. Pixell-Goodrich's paper on "The Determination of Age in Honey-bees.

[The figures were drawn with the aid of a camera lucida at an approximate magnification of 2000 unless otherwise stated.]

Ant. Antennal nerve proceeding from antennal lobe of brain. *c.* Commissures. *E.* Compound eye. *e.* Simple eyes. *lab.* Labial nerve. *max.* Maxillary nerve. *md.* Mandibular nerve. *n.c.* Ventral nerve-cord. *œs.* Œosphagus. *œs. gl.* Œsophageal glands. *phar.* Pharynx. *s.g.* Sub-œsophageal ganglion.

Fig. 1.—Front view of the whole brain after removal of the pharyngeal glands. × 20.

Fig. 2.—Right postero-lateral view of brain showing alimentary canal with œsophageal glands. × 23.

Fig. 3.—The right half of a section through the lower part of sub-œsophageal ganglion of a young bee immediately after hatching, showing the central mass of ganglion-cells between the fibres going to the mandibular, maxillary and labial nerves. Orth's alcoholic carmine and picro-nigrosin. × 400.

Fig. 4.—A corresponding half section through the same ganglion of a bee five or six months old after hibernation, showing the necrotic condition of the cells. Iron-hæmatoxylin and orange G. × 400.

Fig. 5.—A large cell from the subœsophageal ganglion of an imago immediately before hatching, showing its general plump appearance

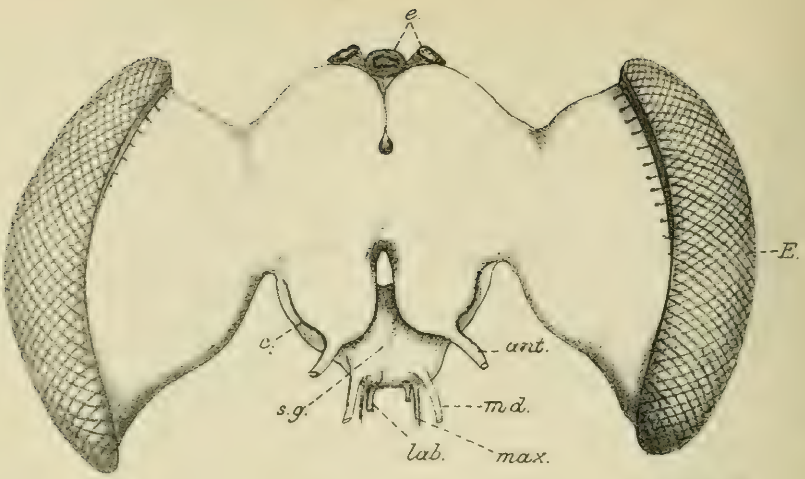
and freedom from highly chromatic substance in the cytoplasm. Iron-hæmatoxylin and picro-nigrosin.

Fig. 6.—A similar cell from the subœsophageal ganglion of a bee seven days after hatching showing the periphery of the cytoplasm already becoming slightly vacuolated. Ehrlich's hæmatoxylin and orange G.

Fig. 7.—A corresponding cell from a foraging bee on returning to the hive laden with pollen. The cytoplasm is decidedly vacuolated at the periphery. Ehrlich's hæmatoxylin and eosin.

Fig. 8.—A corresponding cell from a bee unable to fly, apparently senescent. Ehrlich's hæmatoxylin and orange G.

Fig 9.—A corresponding cell from a bee dying in March after hibernation. Iron-hæmatoxylin and orange G.



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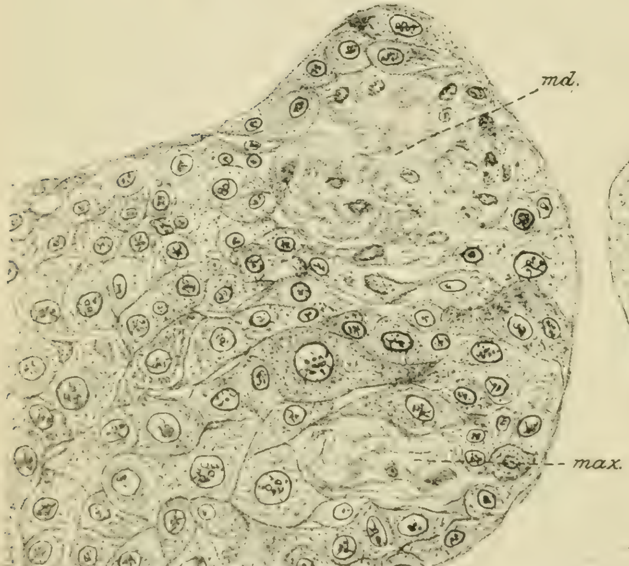

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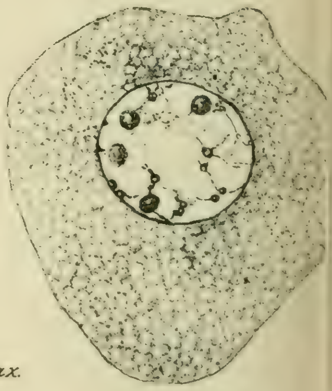

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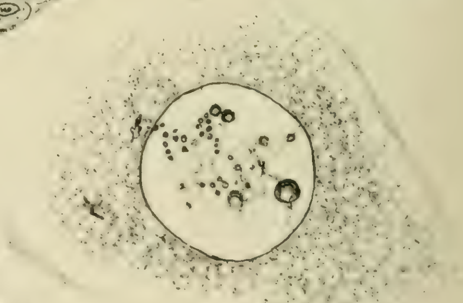

Fig. 6

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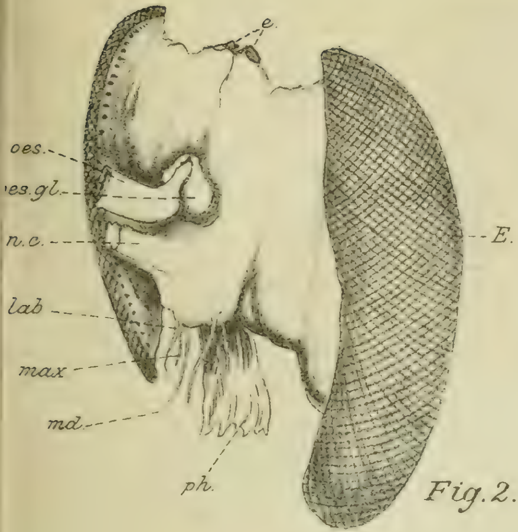

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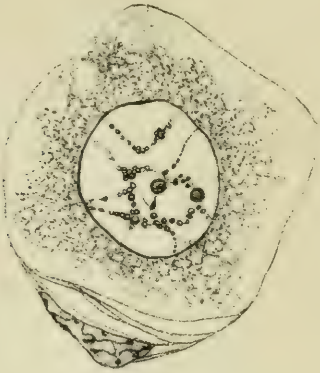

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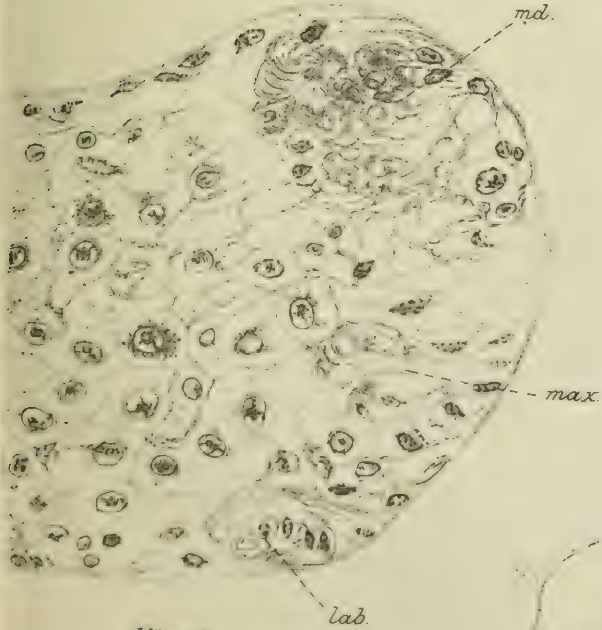

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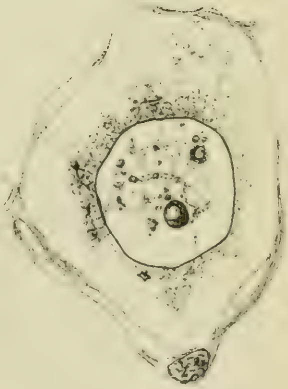

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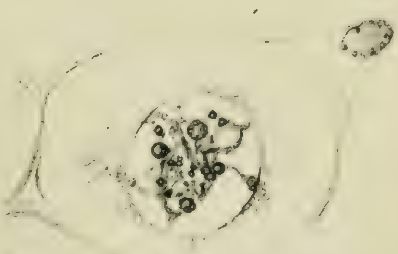

Fig. 9.

Huth, London.

The Chromosome Cycle of Gregarines, with Special Reference to Diplocystis schneideri Kunstler.

By

A. Pringle Jameson.

With Plates 12 to 15.

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INTRODUCTION.

ALTHOUGH numerous papers have been published on the life-histories of Gregarines, no one has, up to the present, attempted to study in detail all nuclear phases in a single life-cycle. As a rule the earliest and the latest stages have received but scanty attention, while the intermediate ones have been unduly stressed in consequence. Our knowledge of this group is thus, in this respect, lop-sided and scrappy. On certain points a considerable body of facts is available, but the lack of any connected thread has prevented their real value from being appreciated; for, in order that the different stages in a life-history may be properly understood, it is obvious that all the stages must be known.

The study of Diplocystis schneideri was undertaken to try to supply the missing thread in at least one gregarine life-cycle. My intention was to study every nuclear phase with care. This particular parasite was selected on account of its large size, and also because a constant supply of material could be assured. It was intended to supplement the work done on Diplocystis by the investigation of other Gregarines: but unfortunately my work was interrupted by the war, and had to lie untouched for over four years. When at last I could resume my researches the time at my disposal was very limited. I therefore confined myself to finishing my work on Diplocystis, leaving the other Gregarines for some future occasion.

In this paper I shall concern myself chiefly with the nucleus and the various nuclear changes. The general morphology of Diplocystis is not of particular interest or importance, and I shall touch that side of the subject only briefly.

My work on Diplocystis schneideri has been carried out, to a great extent, in association with Mr. Clifford Dobell, to whose researches—still, for the most part, unpublished—on the coccidian Aggregata eberthi it forms a counterpart. In an earlier joint paper (Dobell and Jameson, 1915) we have already recorded the chief results of our work, in so far as they concern the behaviour of the chromosomes throughout the life-histories of these two Sporozoa. It will be convenient to state very briefly here what these results were. In the first place, we found that our observations were in complete agreement in showing that the nuclear divisions, at all stages in the life-cycles of the organisms studied, are mitotic. Amitosis, resolution into chromidia, disappearance of the nuclei at certain stages—all of which have been described in the Coccidia and Gregarines—do not occur during normal development. Secondly, we found that the number of chromosomes formed during mitosis is constant. The chromosomes are neither indefinite nor variable in number, but as definite and constant as in any of the multicellular animals and plants. Thirdly, we found that both the organisms studied showed, with the greatest clearness, that the chromosome cycle is remarkable, differing considerably from that which, on analogy with the Metazoa, previous workers had been led to expect. We found that the nuclei of Aggregata and Diplocystis contain, at every stage in the life-history save one, a chromosome complex which consists of a single set of differentiated elements, constituting a haploid—not a diploid—group. The one exception is the zygote nucleus, which is formed, as in other organisms, by the fusion of two gamete nuclei, each containing a single (haploid) set of chromosomes. The zygote nucleus is the only diploid nucleus in the life-cycle. In agreement with these findings, we found further that reduction, or meiosis—the halving of the chromosome number—occurs immediately after, and not before, fertilization. The first division of the zygote nucleus is a true reduction division, comparable with the reduction divisions which occur in the gametogenesis of the Metazoa.

It is thus clear that the chromosome cycle in Aggregata and Diplocystis—and, in all probability, in other Coccidia and Gregarines—is radically different from that which has been demonstrated in the Metazoa.

In the present paper I shall describe my own observations upon Diplocystis schneideri as briefly and objectively as possible, without reference to the work of others on other Gregarines, and laying particular emphasis upon the behaviour of the nuclei and chromosomes throughout the life-cycle. In the second part I shall then consider the observations of other workers, in so far as they are relevant, and attempt to show how they should be interpreted. All the discrepant facts can, I think, be reconciled in the light of my own findings; and they already tend to show, I believe, that the conclusions drawn from the study of Diplocystis will probably be found to hold good for the whole group of Gregarines in general.

I wish to express my gratitude to my friend, Mr. Clifford Dobell, F.R.S., for the assistance which he has rendered me at every stage of my investigation. Not only did he suggest to me the line of work which I have attempted to follow, but his advice and criticisms have been invaluable to me during its progress. Indeed, if it had not been for his kindly help and sympathy the work might never have been completed.

The work was commenced at the Imperial College of Science while I held the Fullerton Scholarship from Aberdeen University. Its completion, at the same place, has been made

possible by a grant from the Committee for Scientific and Industrial Research, to which body I wish to express my thanks.

PART I.—THE LIFE-CYCLE OF DIPLOCYSTIS SCHNEIDERI, WITH SPECIAL REFERENCE TO THE BEHAVIOUR OF THE NUCLEI AND CHROMOSOMES.

A. Material, Methods, and Infection Experiments.

Diplocystis schneideri was originally described by Kunstler in 1887 from the body-cavity of the ship cockroach (Periplaneta americana). The material on which I have worked was obtained from the same insect host, but I have also found a certain number of the bakehouse cockroach (Stylopyga orientalis) to be infected with the parasite. I was fortunate enough to get an almost unlimited supply of P. americana from the Reptile House in the gardens of the Zoological Society. Of these cockroaches 81 per cent. were found to be infected with D. schneideri.

As is well known, it is not easy to fix gregarine material satisfactorily. In common with most workers I have found that good fixation is obtained only with picric acid fixatives. Picro-acetic, in both aqueous and alcoholic solution, was perhaps the most successful; but the fluid of Bouin, and Duboscq's alcoholic modification of it, were extremely good.

In embedding, the objects were not passed directly from xylol into paraffin, but were passed through slightly warmed baths of chloroform and paraffin and through several changes of paraffin in the bath. The food-granules in the protoplasm were found to cut more easily after this graduated embedding process.

As a rule the parasites were stained very lightly with borax carmine before embedding. They were then inspected with a pocket lens. In this way it was possible to sort the specimens into lots roughly corresponding to the stages in the life-history. For example, those in which the nucleus had not commenced to divide had a prominent nucleus ; those in which the early divisions were taking place had no visible nucleus : those in which the nuclei were collecting round the periphery for gamete formation had a delicate pink zone round the periphery ; those which were flaccid and watery had formed spores. By sectioning a few specimens together from one of the lots, and not sectioning individuals at random, the very laborious work of cutting and searching through many sections for the different stages in the life-cycle was somewhat lessened.

In staining, Dobell's alcoholic iron-alum hæmatein stain was found to be invaluable. In crispness and delicacy of colouring this stain far surpasses any other. Its more general use would, I believe, reveal much that up to the present has been hidden. Heidenhain's iron-alum hæmatoxylin was used to some extent, but did not reveal the finer details. Safranin was also occasionally employed, as was borax carmine for whole mounts.

The spores are extremely difficult to stain. In smears they were only imperfectly stained with acid carmine. No other stain penetrated the spore-wall at all. In thin sections, of course, it was possible to get quite excellent results.

In order to study the dehiscence of the spores I treated them on the slide with mid-gut extract, but this had little or no effect. At times the sporozoites within the spore seemed to move, but the spores never showed any signs of dehiscing. Sixty-five cockroaches were fed on spores. Of these, thirty were examined for dehiscence of the spore by smearing the mid-gut contents on coverslips. In only two of these were the spores found to have opened. The remaining thirty-five were killed at different intervals and the mid-guts were sectioned to find the penetration of the gut by the sporozoites, but in only three cases were young parasites found in the wall.

These experiments are of little value for two reasons. In the first place, since 81 per cent. of the cockroaches used were found to be naturally infected with the parasite, it was impossible to be sure that the young parasites I found were derived from the spores on which I had fed the cockroaches and not from a previous wild infection. In the second place, it is not possible to keep such active animals as cockroaches in captivity under perfectly normal conditions. They eat little, and probably their digestive juices are not sufficiently normal to cause the spores to dehisce.

B. Macroscopic Appearance and Sketch of the Life-history.

The parasites lie freely in the body-cavity of their host. Almost invariably two individuals are found associated together, although occasionally single parasites or associations of three parasites are found. The former can never complete their life-cycle, and of the latter I have found so few specimens that I have been unable to come to any conclusion regarding their development. In the body of a single cockroach there are to be found parasites at several different stages of development, but the vegetative forms are the most common. These show very clearly the two members of the association (Pl. 12, fig. 1). The younger and smaller pairs resemble two tiny seed-pearls stuck together. Each parasite is rounded in outline, being only slightly flattened at the point of contact with its fellow. They are chalky white in colour. The smallest forms that are found in the body-cavity are easily visible to the naked eye. As growth proceeds the point of contact between the two members of a pair becomes more flattened and larger, but the dual origin of the association is always clearly seen during the vegetative life. The parasites grow to a comparatively large size, a length of 2 mm. or even a little more for the long axis of the pair being not uncommon.

Gradually the pairs lose their original appearance of two little pearls pressed together, becoming oval, but slightly constricted in the middle. Finally the constriction disappears and they become oblong-oval in shape. At the same time a clear zone appears round the periphery of each parasite—a sign that the reproductive period has been entered upon.

The clear zone next disappears and the association loses its pure white colour and becomes watery white. While this change has been going on the "cyst"—for so one can now speak of it—becomes flaccid, the contents becoming very fluid, whereas in the vegetative forms the parasites have very firm bodies.

These stages, as will be seen later on, are associated with spore-formation, the flaccid, watery cyst being filled with spores. Unless these spores are eaten by a cockroach no further development takes place, and the cysts gradually dry and shrivel up, finally becoming withered-looking little brown bodies. If, on the other hand, the host should die or be killed when the spores are ripe, it will almost invariably be eaten by some of its fellows. The spores then dehisce in the gut of the new host, and the liberated sporozoites penetrate the gut-wall, where they associate, evidently quite fortuitously, in pairs. They grow in size, lying immediately under the basal layer of the gut-wall, until at last this ruptures and they fall into the surrounding body-spaces.

Although the parasites may be present in such numbers that every available space inside the body-walls is literally packed tight with them, the host appears to suffer no inconvenience. In particular I examined the reproductive organs to determine whether they had been affected in any way, but even in the most heavily infected cockroaches no sterility seemed to have been produced. In certain of the guts examined, in which numerous parasites were found lying in the walls, the basal membrane showed a very considerable thickening.

c. The Spore and the Sporozoites.

I propose to commence my detailed account of the life-history of Diplocystis schneideri at the point where a cockroach becomes infected with this parasite. Infection is accomplished by a cockroach eating the spores, which, from the nature of the parasite, must be contained within the body of another cockroach. As I have already pointed out, the spores of D. schneideri are formed within the body-cavity spaces of the cockroach when the parasite has completed its life-cycle. There is, of course, no exit for the spores from these spaces; but as cockroaches are cannibals, and practically always gnaw the abdomen of dead or dying comrades and eat out the abdominal contents, they will almost certainly be eaten by another cockroach. When a high percentage of cockroaches is infected, some of those that are ready to serve for a meal for the others are almost certain to contain ripe spores of Diplocystis.

The spores are 7 to 7·5 μ in length, and oval in shape (Pl. 12, fig. 2). The spore wall is thick, but there is a well-marked, Y-shaped line of dehiscence (Pl. 12, fig. 3), so that when the wall ruptures along this line there is formed a triangular opening through which the sporozoites escape (Pl. 12, fig. 4). Within the spore are eight sporozoites, lying coiled up in such a way that only four can be clearly seen at once. As the spores stain very badly it is difficult to make out the finer details of their arrangement within the wall.

Dehiscence takes place in the mid-gut. It takes thirty hours for ingested materials to pass through the crop and gizzard of the cockroach and down to the end of the mid-gut,[1] and thus the spores are subjected to a prolonged soaking process in the crop before the action of the digestive juices of the mid-gut finally causes them to open.

---

[1] In order to determine how long it took for food to reach the mid-gut, a number of cockroaches were heavily fed on carmine mixed to a paste with sweetened water. The cockroaches were killed at different intervals of time and their guts were examined. The carmine showed up very distinctly through the thin gut-wall, and one could thus find out easily how far down the gut the carmine meal had got in the given time. I found thirty hours was the time taken, in all cockroaches examined, for food to pass from the mouth to the hind end of the mid-gut. Of course, some of the meal was longer in this part of the gut, as it seems to pass rather slowly through the gizzard, but by thirty hours an appreciable quantity of the meal had passed through.

The sporozoites are some 8 μ in length (Pl. 12, fig. 5). They are roughly spindle-shaped, but tapering more towards one end than the other. Kunstler (1887) describes them as being "club-shaped," thus emphasizing the fact that one end is somewhat thickened. They have a rounded, rather plump appearance. The nucleus, which is situated close to the thicker end, is very small—about 1 μ long—and looks like a tiny vacuole slightly drawn out in the long axis of the sporozoite. At either end of the nucleus there is a small granule of chromatin, and the whole structure thus has the appearance of two tiny chromatin caps separated by a little clear vacuole. The sporozoites advance by a writhing movement (Pl. 12, fig. 6), with the more slender end anterior, the nucleus marking the posterior. I was unable to see any sign of the amœboid process of which Kunstler speaks.

D. Penetration of the Gut-wall and Growth of the Parasite therein.

The sporozoites penetrate the gut-wall in the posterior part of the mid-gut, just in front of the origin of the Malpighian tubes. I have never found any parasites in the anterior half of the mid-gut wall. They penetrate between the long columnar cells of the epithelium, and rapidly make their way through this layer until they come to the basal ends of the cells, where the nuclei are situated, immediately under the basal membrane (Pl. 12, fig. 7). The parasites remain here, and commence their growth.

For a short time the spindle shape of the sporozoite is retained, the posterior end becoming stouter, but without much growth in length taking place (Pl. 12, fig. 8). The posterior end increases most rapidly and becomes at first flattened and then more rounded (Pl. 12, figs. 9 and 10), a few specimens at this stage showing rather an irregular outline, as if the organisms were somewhat amœboid (Pl. 12, fig. 9). While the posterior portion is increasing greatly in size the anterior portion may remain more or less pointed, so that the young parasite becomes lanceolate in shape (Pl. 12, figs. 13 and 14). Soon, however, all trace of the original spindle form is lost and the parasite becomes oval or roundish (Pl. 12, figs. 10, 16–19). While growth in size is proceeding the cytoplasm becomes very much vacuolated. The larger the parasite the more it is vacuolated (Pl. 12, figs. 12, 14, 16), although the sporozoites and the very young stages in the gut-wall show hardly any vacuolation of their cytoplasm.

The behaviour of the nucleus during this period is of the greatest interest. As soon as the parasite commences to grow, the anterior chromatin particle in the nucleus also increases in size. It forms a round body which stains much less deeply with chromatin stains than the posterior particle (Pl. 12, fig. 11). By its rapid growth the space separating the anterior from the posterior nuclear cap is abolished, so that the posterior cap, which does not at first grow appreciably in size, comes to lie in close contact with the now very large and palely staining anterior cap, which may be called the nucleolus. Although the nucleus is almost completely filled by the large nucleolus, careful inspection always shows that there is a small clear area limited by a delicate membrane surrounding part of the nucleolus (Pl. 12, fig. 12). The nucleus is now many times larger than the sporozoite nucleus.

At first the darkly-staining posterior chromatin granule lies closely pressed against the nucleolus. Soon, however, it is seen that it is actually making its way inside the nucleolus. In the first stages of this process the darkly-staining portion, which I propose to call the micronucleus for reasons which will be discussed later, grows somewhat in size and sends a rounded projection into the nucleolus, so that the micronucleus becomes hour-glass-shaped (Pl. 12, fig. 13). That portion of the micronucleus which projects into the nucleolus then moves still further inwards (Pl. 12, fig. 14), though it seems to remain connected with the outside portion by a delicate strand or tube. Finally, the external portion also moves gradually inside the nucleolus (Pl. 12, fig. 15), so that the entire micronucleus ultimately lies within it as a round, darkly-staining body (Pl. 12, fig. 16). While this process has been taking place, the nucleolus has become slightly vacuolated (Pl. 12, figs. 14–16).

At first the micronucleus seems to be a homogeneous body, but soon vacuoles begin to appear in it (Pl. 12, fig. 17). It grows in size and becomes slightly paler in staining reactions owing to the presence of vacuoles, but it always stains much more deeply than the surrounding nucleolus (Pl. 12, figs. 17–19). The small clear area already noted as occurring in the early nucleus grows in size very rapidly, and soon surrounds the nucleolus with a large nucleoplasm area bounded by a very delicate membrane (Pl. 12, figs. 14–16). In fixed and stained specimens a delicate network can be made out in it, but I believe this is due to fixation.

The relatively large nucleus thus formed therefore contains within it a large nucleolus inside which is a micronucleus. I propose to call this structure of dual origin the karyosome.

The nucleus does not long remain in this simple form. The nucleolus becomes increasingly vacuolated and irregular in shape, blunt projections which are ultimately "budded" off being formed round the periphery (Pl. 12, fig. 20). The micronucleus also becomes much more vacuolated and irregular in shape (Pl. 12, fig. 21). At times the micronucleus seems to bud off chromatin masses (Pl. 12, fig. 22), but I have not often observed this, and I am uncertain whether it is a normal process or not. Finally the karyosome breaks up and gives rise to a large, much vacuolated portion evidently derived from the micronucleus, as it stains in much the same way, and several less vacuolated, more palely-staining fragments (Pl. 12, fig. 23). These last disappear and the large micronuclear portion breaks up into smaller particles (Pl. 12, fig. 24), which in turn give rise to still smaller fragments chiefly by the growth of vacuoles in them and the budding off of vesicles (Pl. 12, fig. 25). In this way a large number of vacuolated particles are formed in the nucleus, which has all the time been increasing rapidly in size. These particles are of all sizes and shapes, but one or two fairly large and regular pieces are always present. These larger portions seem to be the reserve from which smaller fragments are constantly being formed. There is thus a continual breaking down of chromatin material going on within the nucleus.

At the commencement of the fragmentation of the karyosome the cytoplasm is much vacuolated and contains no reserve food-granules. It stains very palely. After the process of chromatin disintegration has been going on for some little time, the cytoplasm round the nucleus begins to stain more deeply in a patchy fashion. Chiefly within the more darkly staining areas of cytoplasm, but also to a slight extent all round the nucleus, small spherical bodies begin to appear (Pl. 12, fig. 26). These are reserve food-granules. They increase rapidly in number and size, until the whole parasite becomes packed full of them, so that the cytoplasm almost disappears from view, remaining only as an extraordinarily delicate network between the food-granules.

While these nuclear changes have been going on the parasites generally associate in pairs. This usually takes place before the formation of food-granules, but there does not seem to be any very fixed time for it. Apparently any two parasites may associate, and there are no visible differences between the members of a pair. At first they lie together, touching only at a small point (Pl. 12, fig. 10), but as the organisms grow larger the surface at the point of contact becomes flattened and correspondingly increased in size. When first they unite, sections show the walls at the edges of the area of contact pressed closely together, and in the middle region fused (Pl. 13, fig. 30). Later the fusion becomes more complete, and one sees a slight thickening all round the circumference of the area of contact and fusion (Pl. 13, fig. 31), while the centre of this area is very thin. There is no thick cyst-wall formed round the two parasites, but the thickening at the circumference of the area of fusion and the presence of a delicate membrane round the two associates may correspond to the cyst-wall of other gregarines.

It may be noted here that not infrequently solitary forms of Diplocystis schneideri are met with. These are frequently quite large, but they never complete their development. They are usually found in cockroaches which are not heavily infected with parasites. Occasionally, too, the young parasites make their way inside older ones, but they never advance beyond a very early stage in development (Pl. 13, fig. 29).

The parasite has now completed its growth in the gut-wall, and as it increases in size it bulges into the body-cavity (Pl. 13, fig. 27). Finally the basal membrane ruptures and the parasites become free in the body-spaces of the host. It may happen that as the parasite grows in size it bulges more into the gut than into the body-cavity (Pl. 13, fig. 28), and it is thus possible that some at least may fall into the gut, and, passing to the outside, fail to complete their development.

E. Growth in the Body-cavity.

It will be well at this point to describe a typical Diplocystis pair. The two associates are approximately equal in size (Pl. 12, fig. 1). They are rounded in outline with the surface of contact between the two flattened. Each parasite is surrounded by a delicate membrane (Pl. 13, fig. 31) outside which is a well-marked wall. This wall, which is practically absent in the area of fusion between the parasites, is alveolar in structure (Pl. 13, fig. 31). To the outside of this again there is another very delicate membrane surrounding the two associates and not running into the partition between them. In a section one can see very clearly the thickening which fills the triangular space between the wall of each parasite and the outer membrane at the circumference of the area of contact. Each parasite is filled with spherical food-granules. These stain with hæmatoxylin; they are coloured brown in iodine solution, turning purple on the addition of sulphuric acid; they are soluble in strong, but insoluble in dilute mineral acids, in acetic acid and in alcohol. Judging by these reactions the granules can be called paraglycogen, but that does not really throw much light on what they are. It is very difficult to see the cytoplasm among the crowd of reserve food-stuff.

The nuclei are relatively large and much alike in the two associates. The nuclear membrane is delicate, and chromatin is present in vacuolated "karyosomes" and small particles (Pl. 13, figs. 32 and 33). All through the nucleus is an irregular network—perhaps caused by fixation—and on this network there is a fine dust of chromatin. The appearance of Pl. 13, figs. 32 and 33, is very characteristic. The larger chromatin particles occupy roughly the centre of a circular area in which the irregular linin network can be seen, while round the periphery of the circle are the small chromatin particles, and outside this again is the network sprinkled with the chromatin dust. These appearances suggest that the larger pieces are giving off smaller portions, which in turn break down into the finest particles. The number of large "karyosomes" is very variable. Sometimes there is only one, sometimes there are several, but I have never seen them absent altogether.

The parasites grow in the body-cavity. How long they remain there before proceeding to gamete formation I am quite unable to say. They pass gradually from the vegetative to the reproductive phase so that it is impossible to draw any line between the two stages.

F. The First Nuclear Division of the Adult Parasite (Gamont).

The first indication of nuclear division is a decrease in the number of small, irregular chromatin fragments in the nucleus. This is accompanied by an increase in the amount of fine chromatin dust (Pl. 13, fig. 34). At the same time one notices that the karyosomes are represented by regular, delicate, vacuolated vesicles, one of which is usually larger than the others. During this stage, and indeed for some time previous to it, the outline of the nucleus in fixed specimens is very irregular. This is due, I believe, to the nature of the contents of the cyst—a large, relatively soft nucleus with a very delicate wall surrounded by a mass of hard food-granules with very little cytoplasm among them.

In giving an account of the first division of the nucleus it is unwise, I think, to do more than indicate the different phases found and to describe these as separate stages. No one has yet been so fortunate as to obtain in any Gregarine a series of figures complete enough to give a detailed description, and no little obscurity is caused by glossing over gaps instead of admitting them.

Prophases.—I have found only five early stages in the first division. They are shown in Pl. 13, figs. 35–41. Pl. 13, fig. 35 shows a nucleus in which, apart from the discrete chromatin dust, there are eight tiny particles of chromatin, each composed of one, or at most two, tiny vesicles. At a point on the periphery[1] there is seen a single small aster. It will be noted that the nuclear wall at this point has broken down. Pl. 13, fig. 36 shows the aster more highly magnified. The rupture in the nuclear membrane has allowed some of its contents to escape, and the aster is lying in the middle of this area. The aster has a dense centre with a rather diffuse outer zone.

Pl. 13, fig. 35, is the only specimen I have which shows several small chromatin vesicles, for usually there is only one large one (Pl. 13, figs. 37–40). Pl. 13, fig. 39, is typical. There is a large, very delicate chromatin vesicle seen in optical section. It will be noted that the wall of the vesicle is very thin in one hemisphere while the opposite hemisphere is thicker and vacuolated. Pl. 13, fig. 37, shows a surface view of a similar karyosome in which the thicker-walled hemisphere is budding off a vesicle. In Pl. 13, fig. 39, too, there is seen an aster which is actually dividing into two, but as this is viewed from above and can only be found by focussing up and down, it is impossible to represent it in a drawing. Here again it will be noted that the aster is lying in a small mass of nuclear material which has protruded through the broken nuclear membrane.

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[1] Fig. 35 has been reconstructed from seven sections. In the figure a small lighter area is seen. This represents a very small "shaving" that has been sliced off the nucleus and which is actually in an eighth section. To the right-hand side of this clear area is seen the tiny aster. The nuclear wall is there broken down. It is to this point on the periphery of the small area that I refer, not, of course, to a point on the extreme circumference of the nucleus.

Here I may refer to Pl. 13, figs. 38 and 40. Pl. 13, fig. 38, does not seem to fit into any normal series, for here we have two asters lying some little distance apart. They are outside the nucleus, but are actually lying in nuclear material which has evidently been extruded through the broken nuclear membrane. A rather solid-looking karyosome is seen near the nuclear membrane, and there are four food-granules lying beside and between the asters. Pl. 13, fig. 40, shows an aster lying within the nucleus and close beside a karyosome. It is, of course, impossible to say whether the aster has moved inwards from the periphery to this position or whether it originated there.

In Pl. 13, fig. 41, the origin of the chromosomes is seen. There is a single large "karyosome" in the nucleus, and near the periphery a single aster lying beside a small vesicle of chromatin from which the chromosomes are being derived. Pl. 14, fig. 42, is a more highly magnified picture of this vesicle showing the origin of the chromosomes. It will be seen that the "vesicle" really consists of one larger and two very tiny ones. Three chromosomes are lying above the vesicles. One of them is a slightly twisted beaded filament, and the other two are straight beaded filaments. At one end of each of these two there is a round globule of chromatin. The aster lies slightly beneath the chromatin vesicles. There are two vacuoles present which bulge out the nuclear wall, as if about to burst it.

Metaphase.—My preparations contain only one metaphase stage. This is figured in Pl. 14, fig. 43. The asters are large, with a rather small spindle between, on which the three globular chromosomes are dividing. Two vacuolated fragments of chromatin are lying on the achromatic figure. The division is taking place in débris of the now disintegrated nucleus.

The form of the chromosomes is to be particularly noted. In Pl. 14, fig. 42, they are seen to be filamentar, two of the filaments having thickened ends. It is evident that the chromosomes arise as filaments and then become condensed into spherical bodies. A difference in size is noticeable among the chromosomes: the right-hand pair is largest, the middle one intermediate and the left-hand one smallest.

Anaphases.—I have found several anaphases but they are all late. A typical one is shown in Pl. 14, fig. 44. The two sets of three daughter-chromosomes have retreated to the poles of the spindle and now lie close up against the asters with a stout centrodesmose connecting the two groups. The size difference in the chromosomes is still evident. It will be noted that as the daughter-chromosome groups have moved apart the spindle has lengthened so that the asters now lie farther apart than in the metaphase.

Telophases.—In the late anaphases a vacuole can be seen forming round the chromosomes, and this is undoubtedly the first sign of the reconstruction of the nuclei. Unfortunately my preparations show no stages between these late anaphases and the late telophase shown in Pl. 14, fig. 45, in which the two daughter-nuclei are fully formed. In each there is a single karyosome and a small amount of chromatin arranged in tiny particles on the nuclear wall and on the linin network. The distance between the two daughter-nuclei is now very considerable. All trace of the primary nucleus has disappeared and the nuclei lie freely among the food-granules. In these young nuclei the asters are still large. Pl. 14, fig. 46, shows an older nucleus from the first division. It has increased greatly in size, those shown in Pl. 14, fig. 45, being about $5\,\mu$ in diameter while this one is about $8{\cdot}6\,\mu$, but the aster is relatively smaller and much more condensed. It persists through the resting stage in this somewhat concentrated form on the outside of the nucleus.

G. The Later Nuclear Divisions.

Second and third divisions are not abundant in my preparations. Pl. 14, figs. 47 and 48, show a metaphase and an anaphase of the second division. Division is taking place in a little web of cytoplasm stretched between the rays of the asters. The three chromosomes are still almost spherical bodies. Three stages of the third division are represented in Pl. 14, figs. 49 to 51—an equatorial plate, an early anaphase, and a late anaphase respectively. The chromosomes are filamentar in the first two, but in the third are stumpy rods. The asters are very large and striking. Three chromosomes are present throughout.

Of the fourth and fifth divisions I have very many stages although the early prophases are rather rare. The aster divides into two (Pl. 14, fig. 52). The chromatin becomes divided into fine particles which distribute themselves over the linin network (Pl. 14, figs. 53–55). Pl. 14, fig. 53, is peculiar. The chromatin seems to be given off from the karyosome, travelling outwards from it along a very definite line. The very loose and irregular spireme which is thus formed (Pl. 14, fig. 55) condenses and segments to form three filamentar beaded chromosomes (Pl. 14, fig. 56). Fibres from the two asters invade the nucleus and come into relation with the three chromosomes (Pl. 14, fig. 57). A spindle is thus formed, with the chromosomes on the equatorial plate, and the nuclear wall disappears (Pl. 14, fig. 58). The three chromosomes split longitudinally (Pl. 14, fig. 59), and then move along the spindle towards the poles (Pl. 14, fig. 60), while at the same time the two asters draw apart (Pl. 14, fig. 61). When the chromosomes reach the poles of the spindle they move right to the heart of the asters and a vacuole begins to form round them (Pl. 14, fig. 62). The chromosomes shorten and become thick at the ends as they lie within the vacuole, and ultimately give rise to several small round chromatin masses (Pl. 14, fig. 63). Finally the greater part of the chromatin becomes concentrated in a single karyosome, a small amount being distributed in tiny granules over the nuclear wall and on the linin network (Pl. 15, fig. 64). A well-marked centrodesmose persists for some time during the telophases, but ultimately the daughter-nuclei lose all connection with each other, the aster persisting as in the case of the first division (Pl. 15, fig. 65).

Owing to the manner in which the spindles lengthen during division, the nuclei, as division progresses, become scattered all through the body of each parasite. After about the fifth or sixth division the nuclei begin to migrate towards the periphery.

H. The Peripheral Divisions Preceding Gamete Formation.

The migration of the nuclei to the periphery is accompanied by the formation of a peripheral zone of cytoplasm free from food-granules. The nuclei on reaching this zone continue dividing rapidly. At first the divisions are similar to the earlier ones (Pl. 15, fig. 66), with the exception of the fact that the nuclear wall persists longer. Three chromosomes are present at each division, as before. The nuclei lie at the very edge of the cytoplasmic zone. As divisions proceed the asters disappear, and cones of attraction, into which the daughter-chromosomes run and there reconstruct the daughter-nuclei, are formed, projecting nipple-like from the periphery (Pl. 15, figs. 67 and 68). Later still, as the nuclei become smaller, they project almost entirely from the surface. With the decreasing size of the nuclei the chromosomes become stumpy rods (Pl. 15, fig. 69), and finally little spheres (Pl. 15, fig. 70). Division now takes place entirely within the nuclear membrane. A rough spireme is formed (Pl. 15, fig. 71) which gives rise to the globular chromosomes, lying on a spindle which practically fills the nucleus (Pl. 15, fig. 72). The chromosome plates pull apart, and at the same time the spindle pushes the nucleus out into an oblong-oval shape, with the chromosomes at each end (Pl. 15, fig. 73). A division line is formed down the middle of the old nucleus and two rounded daughter-nuclei result lying close together (Pl. 15, fig. 74).

In this way the peripheral cytoplasm zone is soon furnished with a dense pile of nuclei (Pl. 15, figs. 75 and 76). In the resting nucleus the chromatin is in the form of a cap which covers its outer pole (Pl. 15, figs. 74 and 76), the inner portion being quite clear and structureless. Each nucleus is rather more than a hemisphere, attached to the peripheral zone of cytoplasm by a flattened surface.

I. The Formation of the Gametes.

By the time this stage has been reached the partition separating the two associates has broken down. A considerable amount of the food reserve has disappeared, so that the paraglycogen granules are not so tightly packed together. The gametes are formed by the growth of the nuclei outwards from the peripheral layer of cytoplasm, the nuclei drawing out behind them a spindle-shaped body of rather vacuolated protoplasm. The nucleus of the gamete thus lies at the outer or anterior end. The posterior end remains attached to the peripheral cytoplasm zone at first by a thick neck, but as the gamete continues to grow the attachment becomes more slender, until it resembles rather a fine stalk or tail (Pl. 15, fig. 77). The nucleus becomes smaller in size, and the chromatin, which was at first in the form of a cap, becomes more distributed over the nuclear wall and linin network, which has now appeared. The anterior end of the gamete becomes pointed, but it is much more obtuse than the tapering posterior end.

When the gamete is fully formed the attaching stalk becomes exceedingly slender, and, severing its connection with what remains of the peripheral cytoplasm, forms a short, slender "tail"—it can scarcely be called a flagellum (Pl. 15, fig. 78). The fully-formed gamete is club-shaped, with its nucleus lying in the pointed anterior end. The nucleus is large, and contains a considerable amount of chromatin arranged in granules round the periphery and on an achromatic net. The gametes of the two associates appear to be exactly alike.

J. The Union of the Gametes.

The gametes now unite in pairs. It may be noted that it appears as though there was a migration of gametes from one associate into the other, because conjugating gametes are found more abundantly at one end of the cyst than at the other. They first become united by their cytoplasm, later drawing in the tail portion and becoming rounded up (Pl. 15, figs. 79 and 80). Complete fusion of the cytoplasm then takes place, resulting in the formation of a zygote with the two gamete-nuclei lying in its cytoplasm (Pl. 15, fig. 81). The chromatin granules of the nuclei then draw away from the nuclear membranes and collect in the middle of the nuclei (Pl. 15, fig. 81), which at first lie close together, but ultimately fuse. Within the zygote nucleus thus formed the two little clumps of chromatin granules are at first separate (Pl. 15, fig. 82), but later they unite to form a large mulberry-like karyosome (Pl. 15, fig. 83) which contracts subsequently to form a more compact body (Pl. 15, fig. 84). The zygote is now a sporoblast.

K. The First Division (Reduction Division) of the Sporoblast Nucleus.

The karyosome commences to break up. Round particles are given off from it, which seem to move outwards along the achromatic strands towards the periphery of the nucleus (Pl. 15, fig 85). The chromatin particles become arranged on a long thread coiled round the inside of the nuclear wall (Pl. 15, fig. 86). The particles are at first of two sizes—large particles and much smaller ones. The chain then begins to contract towards the centre of the nucleus and the particles become more uniform in size (Pl. 15, fig. 87), until finally a spireme is formed like a coiled string of beads (Pl. 15, fig. 88). The spireme contracts into a tangled knot towards one side of the nucleus, which is frequently drawn out into a long oval at this stage (Pl. 15, figs. 89–91). Then the knot begins to open out (Pl. 15, fig. 92). As the tangle becomes less obscure one can see that the spireme during this synapsis has become divided into six chromosomes (Pl. 15, figs. 93–95). These are as a rule rather difficult to make out, Pl. 15, figs. 93 and 94 being typical. Pl. 15, fig. 95, in which the chromosomes are widely separated, is not common. The chromosomes are in two sets of three—two long, two intermediate, and two short, the last two pairs being very nearly equal in size.

A very faint indication of a spindle arises within the nucleus (Pl. 15, fig. 96), and the chromosomes separate into two homologous groups of three, and then move to the opposite poles of the spindle (Pl. 15, fig. 97). The daughter groups move apart until they reach opposite poles of the sporoblast (Pl. 15, fig. 98), and at the same time the chromosomes shorten and thicken. A vacuole arises around them, and two daughter nuclei are reconstructed, the chromosomes condensing to form a large karyosome in each (Pl. 15, fig. 99).

L. The Later Divisions within the Spore.

Owing to the small size of the nuclei the final divisions in the sporocyst are difficult to make out in their earlier stages. The two daughter-nuclei form spiremes by the breaking up of the karyosomes (Pl. 15, fig. 100), and then give rise to three chromosomes which divide to form daughter-groups (Pl. 15, figs. 101–103). The chromosomes are short rods. Four daughter-nuclei are reconstructed (Pl. 15, fig. 104). These in turn give rise to rough spiremes (Pl. 15, fig. 105), each forming three globular chromosomes which again divide (Pl. 15, figs. 106-108), and eight nuclei thus result.

The sporozoite nuclei are formed from the eight nuclei of the sporoblast. The chromatin is first peripherally placed (Pl. 15, figs. 110 and 111)[1], but it becomes concentrated more and more into two granules which lie one at each end of the oval nucleus (Pl. 15, figs. 109 and 110). The cytoplasm in the sporocyst becomes divided, in the usual way, into eight sporozoites round the eight nuclei.

The formation of the spore-wall is difficult to make out in stained preparations mounted in balsam. It is evident in the late eight-nucleate cyst and appears to begin to form during the last division.

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[1] These two figures, containing only four nuclei and rather smaller in size, are "chips" of rather longish oval sporoblasts in the eight-nucleate stage.

PART II.—THE NUCLEAR PHENOMENA DESCRIBED IN OTHER GREGARINES, CONSIDERED IN THE LIGHT OF THE FOREGOING OBSERVATIONS ON DIPLOCYSTIS.

A. General Remarks.

Having described the behaviour of the nuclei during the life-cycle of Diplocystis schneideri in so far as I have been able to investigate it, I wish now to compare my findings with those of other workers in the same field. Unfortunately most of the work on gregarine life-histories is extremely scrappy. So far as I know, no worker has up to the present attempted to follow in detail the behaviour of the nucleus through every stage in the life-cycle of any Gregarine. On some points, such as the question of the similarity or dissimilarity of the gametes, our knowledge is very full, but on the majority of questions that seem to me to be of interest and importance the information available is lamentably scanty. I shall therefore attempt to combine all the scattered observations, and so construct a more or less connected account of the gregarine nuclear cycle.

In reviewing this work it will be unnecessary to pay any attention to the papers published prior to 1899, in which year Siedlecki's most excellent account of Lankesteria ascidiæ appeared. This investigation outlined the course of gregarine development in a masterly fashion and left only the details to be filled in. During the twenty succeeding years many papers on Gregarines have been published, but with certain outstanding exceptions they have not advanced our knowledge of the subject from the position in which Siedlecki left it. I have read, I believe, all the papers published on gregarines during these years, and if I do not refer to some it will be because they contain little or nothing of importance.

As the Schizogregarines are so very imperfectly investigated I have confined my attention to the Eugregarines. These form a very homogeneous group, and I believe that when they have been more fully studied their life-cycles will be found to be of a very uniform type. At all events a comparison of the extremely imperfect records at present available shows far more of resemblance than of difference.

B. The Early Development of the Nucleus.

Very little attention has been paid by most workers to the early stages of gregarine development. Léger and Duboscq (1902 and 1904) have investigated a number of different forms, but no one of them has been studied in much detail. In all cases the sporozoite nucleus is of the type described for D. schneideri, with peripheral chromatin concentrated in two, usually polar, caps. A single central karyosome is subsequently formed in a way which is not clearly described, but the final result is that the peripheral chromatin disappears, being apparently transferred from the periphery to the karyosome. The karyosomes thus formed are frequently composed of two parts. Thus in Stylorhynchus oblongatus and Pyxinia möbiuszi there is a central more deeply-staining area, just as in Diplocystis schneideri. In Diplocystis major the same thing is found, their fig. 8 (Léger and Duboscq, 1902) being remarkably like my Pl. 12, fig. 21. In connection with this apparently dual nature of the karyosome in gregarines it may be noted that Hesse (1909) mentions certain species of Monocystis which show the same sort of thing.[1] I would call attention particularly to figs. 132 and 135 in Hesse's work, showing the nucleus of Rhynchocystis pilosa with a karyosome having in its middle "une masse irrégulière, vacuolaire, fortement colorée par le fer."

The only account which gives a detailed description of the origin of the karyosome is that of Schellack (1907) for Echinomera hispida. Here the sporozoite nucleus is unlike that of most other gregarines, its chromatin being more or less evenly distributed around the periphery. A thickening of chromatin then appears at some point on the periphery, and in close connection with this a small internal vesicle arises, into which the chromatin of the thickening migrates in the form of small particles. The vesicle finally becomes completely filled with chromatin and stains a uniform deep black with iron-hæmatoxylin.

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[1] "En générale, le karyosome est vacuolaire, et il présente à sa surface une croûte dont la coloration diffère de celle de la moelle."

The evidence before us is not abundant, but there is enough to show that in young gregarines the karyosome is not a simple structure. As a rule it contains within it a chromatin body which I have called the micronucleus. The karyosome, further, is formed within the young nucleus in a peculiar fashion. In Diplocystis schneideri a plastin nucleolus first arises, and later the micronucleus migrates into its interior. It is possible that Schellack's vesicle is also a nucleolus of this sort, and that more delicate staining would reveal that his "karyosome" is not a homogeneous, deeply-staining body, but contains, as in many other gregarines, a micronucleus which migrates into the nucleolus in small particles—as described by Schellack—from the peripheral chromatin thickening.

The true interpretation of the structure and dual origin of the karyosome cannot yet be given. I have called the chromatin body within the karyosome a micronucleus because I believe that it contains the chromatin from which the chromosomes of the first division are derived. Owing to the fragmentation of the karyosome it has been impossible in Diplocystis to follow the history of the micronucleus continuously from its origin to the formation of the chromosomes, but, as I have noted above, there is a hint of continuity in the regular chromatin vesicles which are always to be found in the nucleus among the irregular fragments of karyosome débris. An investigation of some gregarine in which the karyosome does not fragment so early or so completely as in Diplocystis would probably yield important results.

I am confirmed in this interpretation by the evidence afforded by Aggregata eberthi. This coccidian[1] has been investigated in its asexual cycle in the crab, Portunus depurator, by Léger and Duboscq (1908). In this they describe the formation, in the nucleus of the young schizont, of a plastin nucleolus beside which there arises a condensation of chromatin. This karyosome, as the authors call it, makes its way inside the nucleolus and gives rise to "un véritable noyau intranucléolaire qui constituera la couche médullaire du nucléole." My friend Mr. Clifford Dobell, F.R.S., has shown me his beautiful preparations and unpublished drawings of Aggregata eberthi, and he has most kindly allowed me to make the following statement about them. The "karyosome" is formed, much as Léger and Duboscq describe it, not only in the asexual cycle but also in the young male and female forms. The most interesting point, however, is that the chromosomes are eventually formed—in both the sexual and asexual forms—from the micronucleus, which gives off into the nucleoplasm the material from which the chromosomes of the first nuclear division are directly derived.

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[1] Dobell (1914) has shown that Aggregata is a coccidian and not a gregarine.

c. The Behaviour of the Nucleus in the Later Vegetative Stages.

In most Gregarines the chromatin of the nucleus is concentrated, during the early stages, in a single karyosome. A small quantity of chromatin may be present in the form of granules on the linin network, but it is never abundant and may be absent. In many forms the karyosome fragments early in development, while in others it seems to remain unfragmented until just before the first nuclear division. The various accounts of the fragmentation or multiplication of the karyosomes differ somewhat in details, but on the whole it may be said that the process is one of budding accompanied in many cases by vacuolization. The small karyosomes thus formed seem to increase in size and give rise to others in the same fashion. Robinson (1910) has given a short account of a rather different phenomenon in

Kalpidorhynchus arenicolæ. Here the karyosome is said to be hollow and budding takes place into its interior, the young karyosomes escaping later into the nucleoplasm through a temporary micropyle. Robinson points out that Léger and Duboscq describe a similar method of budding in Aggregata,[1] but Kalpidorhynchus is the only case so far described among the Gregarines. It is an organism which would repay careful study. Schellack (1907) claims that in Echinomera hispida the karyosome multiplies in a peculiar manner: "Im Innern des grossen Einzelkaryosome die helle Grundsubstanz wieder mehr hervortritt, das vorhandene Chromatin sich zu Kugeln zusammenballt und schliesslich austritt, wobei der farblose Restkörper mehr oder weniger vom Chromatin befreit zurückbleibt." Judging by the figures, this seems to be nothing different from the disintegration by budding and vacuolization described above.

In Gonospora testiculi, Trégouboff (1918) found that at an early stage in development the karyosome was hollow, containing a clear vacuole in its centre. The contents of the vacuole were expelled into the nucleoplasm later and there gave rise evidently to a micronucleus. Léger and Duboscq (1911) have observed a similar phenomenon in Porospora portunidarum.

The karyosome in gregarines is undoubtedly the centre of many activities. For aught we know it may preside over all the vital functions of the organism, but it gives no clear indication of this. The extraordinary account given by Drzewecki (1904, 1907) of the waxing and waning of the nucleus and the corresponding building up and breaking down of amyloid bodies must be regarded with the greatest suspicion. Hesse (1909), however, has shown that "during the course of the vegetative life, the karyosome seems to be the seat of numerous metamorphoses, and plays an important part in the vital manifestations of the nucleus." As far as one can judge from Hesse's descriptions and figures the karyosome does not appear to be altered to any very great extent during most of these changes, evidently a change in staining reaction being the most noticeable.

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[1] It is difficult to understand how the budding could take place in Aggregata, for the interior of the karyosome is occupied by the micronucleus. Mr. Clifford Dobell tells me that in Aggregata the buds are really formed on the outer side of the karyosome, and not internally.

In the early stages there is, however, one important period during which—in Diplocystis schneideri at all events—the karyosome gives very clear proof that it is connected closely with an important vital function. I refer to the formation of reserve food-granules. While the karyosome is single and unfragmented there are no food-granules present in the cytoplasm; but soon after the process of fragmentation has begun food-granules begin to appear round the nucleus. In the work of Hesse (1909) evidence of a similar though less striking sort may be found. For example, in Monocystis agilis and Stomatophora coronata granules are given off by the karyosome, and they seem to make their way out of the nucleus into the cytoplasm and there play an important part in the synthesis of food-granules.

These phenomena must not be confused with the so-called "chromidia" which have been frequently described in the vegetative forms of various gregarines. By a careful investigation of Stylorhynchus longicollis and Stenophora juli, Comes (1907) has shown that these bodies are not nuclear in origin, but are formed in the cytoplasm as the result of various metabolic processes.

D. The Behaviour of the Nucleus during Reproduction.

Whatever the behaviour of the karyosome may be during the vegetative life of gregarines, there is no little uniformity in the processes which precede the reproductive phases. This period is practically always introduced by the disintegration of the karyosome or karyosomes. There is thus formed within the nucleus a large quantity of chromatin débris in fine particles and small, usually vacuolated, fragments. In all cases which have been adequately investigated an achromatic figure is formed and division proceeds in the ordinary mitotic fashion.

It may be well at this point to note that there are a few cases on record in which mitosis is said not to occur. All of these accounts are obviously founded on the misinterpretation of poor material, but as they have been made the occasion for the revival of the "generative chromidia" hypothesis in gregarines it will be necessary to consider them briefly. Berndt (1902) describes in Gregarina cuneata, G. polymorpha and G. steini a process whereby the chromatin becomes distributed round the periphery of the nucleus in a very finely granular form. The nucleus itself becomes very irregular in outline ("flame structure"). Its membrane disappears, and the chromatin is then set free in the cytoplasm in little masses which migrate to the periphery of the organism, multiplying in a "primitively mitotic" fashion as they go. Kuschakewitsch (1907), for the same gregarines, describes a still more wonderful history, in which the nucleus seems to dissolve away completely, and there then arises round the periphery of the cyst a layer of cytoplasm which stains deeply with chromatin stains, and within which grains of chromatin appear and finally give rise to many nuclei. An inspection of the figures which illustrate these papers is sufficient to convince one that all the material on which these results are based was extraordinarily badly fixed and stained. The mere fact that the "flame structure" of the nucleus was taken to represent a normal stage in development seems to me sufficient to make one regard the work with doubt; for despite the fact that Drzewecki (1903) claimed to have seen this appearance in a living nucleus, I believe it is produced by the action of fixatives on the nuclear wall, which becomes very thin prior to disintegration. As Cuénot (1901) has pointed out: "Les Grégarines sont très difficiles à bien fixer, le noyau se déforme avec la plus grande facilité et prend un aspect irrégulier (noyau flammé de Wolters)." If a badly fixed or degenerate nucleus is taken as an important stage in development, disaster is bound to follow.

Fortunately Léger and Duboscq (1909) have investigated a number of species of the genus Gregarina, including G. polymorpha, and in every case they have seen early nuclei dividing by mitosis. Further, we have Schnitzler's (1905) account of Clepsydrina (Gregarina) ovata, in which he describes a typical mitosis for the first nuclear division. It is unlikely that within the same genus there would be two totally different methods of forming the gamete nuclei.

Swarczewsky (1910) describes, in Lankesteria sp., a process of gamete-nuclei formation which is almost identical with that described by Kuschakewitsch for the meal-worm gregarines. The figures published with the paper are quite as remarkable as those of Kuschakewitsch, and when one learns that the material was obtained from Turbellarians collected by an expedition to Lake Baikal—evidently no special care having been taken in fixing the worms so as to preserve adequately the contained parasites—one is not surprised at the unfortunate results. It is only astonishing that such material should have been regarded as adequate for an investigation requiring the most careful cytological technique. Here again we are fortunate in having a good account of the behaviour of the nucleus in another member of the same genus. Siedlecki's (1899) account of Lankesteria ascidiæ traces the nuclei from the first division to gamete formation, and in every stage typical division figures are found.

Swarczewsky (1912) has attempted to argue that all gregarines form their gamete nuclei by a process of chromidia formation, the difference between the findings of Kuschakewitsch and Swarczewsky and those of other workers being "more of a quantitative than of a qualitative character." On the one hand many nuclei are formed from a chromidium; on the other only one nucleus is so formed. As we have seen, the supposed origin of the many nuclei from a chromidium is the result of studying degenerate and badly-fixed material, while the "chromidium" from which the single nucleus is supposed to be derived is merely nuclear débris. The question of generative chromidia—at least for the Eugregarinæ—does not really exist. It has been based on error and ought never to have been raised.

Putting on one side, then, these obviously faulty observations,[1] we are left with some nine or ten cases in which a typical mitosis has been observed. The first nuclear division is probably the most difficult stage to study in a gregarine life-cycle. It is extremely difficult to find sufficient material to illustrate the various nuclear phases leading up to and culminating in the division of the primary nucleus; and when any stages are found they are frequently obscured by the débris from the disintegrating karyosomes. In consequence of this, very imperfect accounts of the first nuclear division are given by different workers. Further, when material is scanty there is always the temptation to make the most of it, and to attempt to construct a "series" from only two or three doubtful stages. Palæontologists may be able to reconstruct an extinct monster from a few fragments of skeleton, but it is not advisable for protozoologists to adopt a like method. A gregarine life-cycle is not known from the nuclear divisions which precede gamete formation, nor can the division of a nucleus be adequately described when all the available material shows perhaps only one or two very problematic prophases and an odd metaphase or late anaphase. I am far from under-estimating the difficulties which attend the study of gregarine life-histories, but I cannot help thinking that if a larger amount of better material had been carefully examined in certain cases, very different results would have been obtained.

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[1] Another erroneous case may be mentioned. Woodcock (1906), in his paper on Cystobia irregularis, believes that the early divisions are amitotic. In proof of this a very poor and meaningless figure is published showing two bodies, presumably nuclei, connected by a short strand; and we are told that in an individual containing "about thirty daughter-nuclei of the fourth generation," one nucleus of the previous generation was thought to be in the last phases of an indirect division. Such evidence does not carry conviction.

The only accounts of the origin of the first division figure that are at all satisfactory are for members of the Acephaline gregarines. It is extremely unfortunate that we know so little of the early development of the Cephaline forms. For these there are but five accounts of the early nuclear phenomena—Clepsydrina (= Gregarina) ovata by Schnitzler (1905) and Schellack (1912), Echinomera hispida by Schellack (1907), Nina gracilis by Léger and Duboscq (1903 and 1909), and Metamera schubergi by Duke (1910). Of all these examples, moreover, only in Metamera schubergi has the investigator been able to find more than one or two stages. Thus Schnitzler figures two very early prophases and two metaphases in G. ovata; Schellack, for E. hispida, one very early prophase and one metaphase, while for G. ovata he merely confirms and corrects the scanty observations of Schnitzler; Léger and Duboscq four "stages" in N. gracilis, the interpretation of which is doubtful, and which will have to be considered later.

On the other side, some of those who have worked on the Monocystid gregarines have been more successful, and have obtained, if not complete series of stages, at all events a fair number of representative ones. For practically all our knowledge of the origin of the achromatic figure and the chromosomes of the first division we shall be compelled to resort to the members of this group.

Let us first consider the origin of the achromatic figure. Following the disintegration of the karyosome the next step in the process of division is the appearance of an achromatic figure. As a rule this arises at the periphery of the nucleus as a single, round, achromatic body or aster. Mulsow (1911) always found two asters in Monocystis rostata, and thought it probable that they arose independently and not by the division of one into two; but it seems most likely that he failed to discover the earliest stages. At all events, Cuénot (1901) and Brasil (1905), who also studied species of Monocystis, found a single aster which later divided into two. In Diplocystis schneideri this is also the case, and there is a hint of the same sort of thing in Echinomera hispida, as described by Schellack (1907). In Metamera schubergi Duke found that a large achromatic mass appeared inside the nucleus, and gradually became striated and formed a spindle without asters, but all the early nuclear development of this gregarine seems very peculiar. In the various members of the genus Monocystis the asters seem to arise on the outside of the nuclear membrane. In Diplocystis schneideri the origin of the aster seems to be at the periphery of the nucleus, near the spot where the nuclear wall begins to break down, but always in nuclear material. It seems probable that the achromatic figure is always of nuclear origin, for in the cases in which it does not arise inside the nucleus or in nuclear material it is closely applied to the outside of the nuclear wall.

There has been some discussion as to the presence or absence of a centrosome or centriole during the first nuclear division. The question is one to which I myself do not attach much importance, for I believe that "the centrosome is but a subordinate part of the general apparatus of mitosis and one which may be entirely dispensed with" (Wilson, 1906). The evidence on this point is most conflicting. Some authors—e.g. Hesse (1909) for Stomatophora coronata, and Léger and Duboscq (1902) for Stylorhynchus longicollis—have supposed that a granule found in the cytoplasm near the nucleus, even in very young individuals, represents a centriole; but I think the assumption has little to be said for it. These deeply-staining grains have not been shown to take any part in the division of the nucleus, and behaviour during division is, after all, the only test of a centriole. In the descriptions and figures which we now have of the actual division stages, nothing definite is to be found. Thus Mulsow (1911) could demonstrate no centrosome in the asters of

Monocystis rostrata; indeed, the presence or absence of an aster seemed to him to depend on fixation, so that one might even doubt the quality of his material and cytological technique. In Diplocystis schneideri I have found no trace of a centrosome, and in the later divisions at the periphery even asters are absent. On the other hand, in Diplocystis major Cuénot (1901) found large, massive-looking centrosomes. Schnitzler (1905) in Clepsydrina ovata found centrosomes but no asters. Brasil (1905), unlike Mulsow, found very definite centrosomes in all species of Monocystis which he studied. Finally Duke (1910) found in the first divisions of Metamera schubergi neither aster nor centrosome, although in the later divisions both were present. On the whole it seems to me most probable that in gregarines the centrosome is no very definite organ, and its presence in preparations may be dependent to a great extent on the fixative and stain used.

This much may then be said about the achromatic figure. It arises on the periphery of the nucleus, and is most probably nuclear in origin. The original single aster or centrosome or attraction cone—whatever it may be, and whatever one cares to call it—divides into two, and so forms a spindle. The substance of the achromatic figure persists from generation to generation in the form of a daughter-aster, which remains usually on the outside of each daughter-nucleus during its period of reconstruction and rest, and gives rise in turn to two daughter-asters and a new spindle at the next division.

The origin of the chromosomes is a much more interesting and important question. Léger and Duboscq (1909) express the opinion that the method of formation of these varies according to the species of gregarine, there being at least two possibilities: (1) the first division figure may derive its chromosomes directly from the chromatin of the primary nucleus, or (2) a micronucleus may first be formed from which the chromosomes arise later. Such evidence as we possess does not, I think, support this second view. As we shall see, the few cases which have been at all adequately studied show the formation of the chromosomes from a certain portion of the chromatin of the primary nucleus, without the intermediate formation—at this stage—of a micronucleus.

Let us first consider the "micronucleus." The word has been used very loosely. Cuénot (1901) originally gave the name "micronucleus" to "des grains ou de courts filaments groupés en un petit amas" in Monocystis; but when writing of Diplocystis he applied the same term to a fully organized small nucleus. It is, I believe, to this latter kind of "micronucleus" that Léger and Duboscq apply the name, for they both figure and describe in Nina gracilis "une véritable noyau très claire." There are only two cases of this sort recorded—the two mentioned above. Schellack (1907) compared the vesicle which he found in the prophases of the first division of Echinomera hispida to the "micronucleus" of Cuénot; but he must have referred to the "micronucleus" of Monocystis and not to that of Diplocystis, for the figure published does not show a completely organized small nucleus. Later (Schellack, 1912) he refers to it as "eine Art Kleinkern." I shall discuss the "micronucleus" of Schellack below. In the Diplocystis from the cricket which Cuénot describes, after some very peculiar "prophases" evidently four stages were found—a doubtful metaphase, a late anaphase, a single small nucleus (the "micronucleus"), and a stage with two small nuclei. Cuénot was of the opinion that the "micronucleus" arose in some fashion in the cytoplasm, then followed the metaphase and anaphase, and finally the two small nuclei resulted. At the same time he recognized the possibility that the "micronucleus" might be merely one of two daughter-nuclei, the other having been knocked out of the section by the knife. I feel certain that this is the correct interpretation. Léger and Duboscq (1909) describe a similar "micronucleus" for Nina gracilis. Here, again, only four stages were found—or, at all events, figured. The first shows the primary nucleus containing a small clear area, within which is a little group of chromatin particles—probably really the micronucleus. The second shows the primary nucleus disintegrated, with a small stellate area among the débris within which are a few regularly-shaped portions of chromatin —presumably chromosomes. The third is extremely doubtful, and is merely called "formation du micronucléus" (fig. 11). I confess to being able to make out little or nothing in this figure except a rather clearer circular area in the nuclear débris. The fourth figure shows a perfectly formed nucleus with a centrosome. The interpretation seems to me to be the same as for Cuénot's "micronucleus"; it is merely one daughter-nucleus derived from the first division, the other having been overlooked or having dropped out of the section. The figure showing the stellate area with chromosomes is probably really an equatorial plate viewed from one pole of the spindle. There is a point in connection with these so-called "micronuclei" which neither Cuénot nor Léger and Duboscq seem to have noticed. It is this: Supposing these nuclei really do represent a stage between the disintegration of the primary nucleus and the formation of the chromosomes, then before the chromosomes can be derived from such a typical nucleus a series of prophases would have to be gone through. But these have never been found by anyone: and since all these stages are missing, the evidence in favour of the "micronucleus" as described by Cuénot and Léger and Duboscq does not seem to me to be satisfactory. All these cases are thus possibly misinterpretations caused by the lack of sufficient material. It is very probable that the origin of the chromosomes in gregarines takes place in a general way after the fashion now to be described.[1]

As we have seen above, in practically all gregarines the karyosome or karyosomes become broken up into many fragments, some very minute and some larger. It is from some of these that the chromosomes of the first equatorial plate are derived. Most unfortunately very little is known about this extremely interesting stage. Brasil (1905) was the first to publish any observations on the point. In Monocystis he found in his "third type of division" that a round, vacuolated portion of a karyosome gave off numerous small particles which at once formed a spireme and then gave rise to the chromosomes. There is one figure (fig. 1[1]) in Robinson's (1910) account of Kalpidorhynchus arenicolæ which is very suggestive of a spireme, but the whole description is too sketchy to make much of. Mulsow (1911) was fortunate enough, in his work on Monocystis rostrata, to find several stages in the formation of the chromosomes. Here, again, a spireme is formed from what appears to be a round, vacuolated portion of karyosome, the spireme being formed from tiny, rounded pieces of chromatin. A sort of "synapsis" occurs, and the chromosomes form a tangled ball on the plate of the spindle, after which division evidently occurs. In Diplocystis schneideri I have found only one example of the formation of the chromosomes, and here again they are seen to come off in the form of beaded threads from a rounded and vacuolated portion of karyosome, very like the original micronucleus formed in the nucleolus of the young forms. It seems to me almost inconceivable that any fragment of chromatin can form chromosomes in this way. There must surely be some particular portion of chromatin that has this function. As I have already said, I cannot help believing that if it were possible to follow the micronucleus of Diplocystis schneideri through all the nuclear changes from its formation in the youngest parasites up to the stage of the final break-up of the primary nucleus, a complete chain might be established between the chromatin of these early stages and the chromatin which forms the chromosomes. It is at least significant that in those few cases in which the formation of chromosomes has been seen, they all arise in the same fashion from a small chromatin structure of approximately the same form.

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[1] Prowazek (1902) claimed to have seen the nucleus of Monocystis open and give off its contents "in an indistinct striated form" into the cytoplasm. This gave rise to a "micronucleus." As no figures are published these observations must be regarded with the greatest suspicion.

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[1] Duke (1910) has a figure (fig. 15) of Metamera schubergi which is rather like this one, and might almost suggest a spireme; but he failed to find any chromosomes. The whole account of the first nuclear division is very peculiar and difficult to understand. It is quite unlike that of any other gregarine.

I believe one is justified in calling this more or less organized portion of chromatin a micronucleus. There are two cases—apart from the erroneous two already referred to—in which micronuclei have been described, but in which the connection between them and the chromosomes has not been found. I refer to the already mentioned cases of Echinomera hispida and Gonospora testiculi described respectively by Schellack (1907) and Trégouboff (1918). The "micronuclei" in both these differ very much from those of Nina gracilis and Diplocystis major; they are merely tiny vesicles containing small fragments of chromatin. In Echinomera Schellack was able to see the formation of the karyosome, and his figures of the early stages show the "karyosome" to be extremely like the later "micronucleus." Might it not be that, as in Diplocystis schneideri, the micronucleus is formed very early in the life of the organism and remains hidden within the karyosome until just before reproduction, when the disintegration of the karyosome brings it to light again? In Gonospora the micronucleus seems to be formed early in the life-history, being expelled from the karyosome as already described. Before the onset of the first division the micronucleus becomes completely organized, and forms a tiny vesicle very like that of Echinomera. Most unfortunately the formation of chromosomes has not been observed in either of these forms.

At this point I propose to review the rest of our knowledge of chromosomes in gregarines, although in some cases, to which reference will be made, the chromosomes were not studied until the later divisions. I wish first to refer to the chromosome numbers found in different species. The more important of the observations recorded in this connection have been combined in the following table. In many cases no counts have been made by the investigator, but I give in a separate column the numbers shown in the published figures. Even when counts have been made I have also given the numbers shown in the figures, for the two do not always correspond.

Species.	Investigator.	No. of chromosomes given.	No. shown in figures.
Diplocystis major and minor	Cuénot (1901)	—	3
Diplocystis schneideri	Jameson (1915)	3	3
Gregarina munieri	Léger & Duboscq (1909)	—	3
Gregarina ovata	Schellack (1912)	3	3
Uradiophora cuénoti	Mercier (1912)	—	3
Kalpidorhynchus arenicolæ	Robinson (1910)	4	4, many or 3
Monocystis (3 species mixed)	Cuénot (1901)	—	Many, 4 or 3
Monocystis, "2nd type"	Brasil (1905)	—	4 or 5
Monocystis, "3rd type"	,, ,,	—	4–6
Monocystis pareudrili	Cognetti de Martiis (1911)	4–5	4–5
Stenophora juli	Trégouboff (1914)	4, seen only at "reduction"	4
Stylorhynchus longicollis	Léger (1904)	4	4
Echinomera hispida	Schellack (1907)	5	5
Nina gracilis	Léger & Duboscq (1903)	5	5
Urospora lagidis	Brasil (1905a)	8	8
Monocystis rostrata	Mulsow (1911)	8	8 (& ?4)
Monocystis, "1st type"	Brasil (1905)	—	Many
Metamera schubergi	Duke (1910)	No definite chromosomes.	

One cannot fail to notice in this list the remarkable number of gregarines which have an odd number of chromosomes. Léger and Duboscq (1903) were the first to draw attention to this point in their work on *Pterocephalus nobilis* (= *Nina gracilis*). There they found five chromosomes, four short ones and one long one, which they called the axial or unpaired chromosome, and which they found formed the karyosome of the daughter-nuclei after division. Schellack (1907) found the same sort of thing in *Echinomera* hispida. The fifth chromosome was longer than the others and formed the karyosome. He compared it with the accessory chromosomes in insects. Léger and Duboscq (1909) returned to the same subject later and showed that in Nina gracilis the unpaired chromosome forms the karyosome only in part. They also pointed out that Schellack's comparison between the unpaired chromosome in gregarines and the accessory chromosome in insects was not satisfactory, and compared it in turn to a "nucleolar formation." As we shall see later, there is a simple explanation of the odd chromosome numbers found in the gregarines. It seems, indeed, that the "unpaired chromosome" differs in no way from the others except in size, and the size difference may amount to little or nothing; for in Gregarina ovata, in which Schellack (1912) discovered another "unpaired chromosome"—three chromosomes are present—the "odd chromosome" hardly differs at all in size from the two others.

A word may be said about the form of the chromosomes. As a rule they are very definite filamentar, rod-shaped or globular bodies. Often indeed all three different forms are found in one species at different stages of the life-history, as we have seen in Diplocystis schneideri. Occasionally, as in Brasil's (1905) "first type of division" in Monocystis, the chromosomes are represented as many small grains of chromatin. The case of Metamera schubergi, investigated by Duke (1910), is an extreme one of this sort. Here chromosomes are apparently absent, and it is suggested that the chromatin is carried along the spindle-fibres in "discrete particles." The method of nuclear division in this gregarine is so peculiar that its further study is desirable.

When the achromatic figure and the chromosomes have been formed the division takes place in the ordinary mitotic fashion. In many cases the two daughter-groups of chromosomes move apart a considerable distance by the lengthening of the spindle-fibres. In this way the nuclei of the later divisions become well distributed through the cyst. After a number of divisions have taken place all through the cytoplasm the nuclei migrate to the periphery of the cyst. There further multiplication may occur, or there may be only one or two peripheral divisions prior to gamete formation. In most forms the nuclei after the last division proceed to gamete formation, but in some, such as Nina gracilis and Echinomera hispida, the nuclei which are to form the female gametes migrate back from the periphery after multiplication has ceased and form a network through the body of the organism.

Léger (1904) described two types of nuclei, generative and somatic, from Stylorhynchus longicollis. Of these the somatic are the larger, and are thought to have the function of working over the reserve food stuffs and preparing them for the generative nuclei. Nuclei of these two sorts have also been described from Gregarina munieri by Léger and Duboscq (1909), Monocystis sp. by Hoffmann (1909), Stenophora juli by Trégouboff (1914), etc. Others have not observed this nuclear dimorphism, and are of opinion that the large nuclei sometimes found alongside of smaller ones are merely ordinary nuclei delayed in their division (Mulsow, 1911); and it seems clear that these nuclei which get left over and do not develop in time must be incapable of taking part in gamete formation (Brasil, 1905). At present there appears to be no good evidence to show that the nuclei within the cyst are differentiated into the two classes—somatic and generative—postulated by Léger.

E. The Search for Reduction Divisions.

The two divisions immediately preceding gamete formation have received a great deal of attention, for most workers seem to have examined these stages with particular care in the expectation of finding a reduction division after the fashion of the Metazoa. The most varied results have rewarded the investigators, but as a rule nothing really comparable with a true reduction division has been found. Before one can speak of a reduction division having taken place one must demonstrate that the number of chromosomes has been reduced to one half by a process of indirect division. There are only two cases on record which claim to have done this. Before examining these let us note certain other nuclear processes which have been observed.

In certain cases—e.g. Stylorhynchus longicollis, Léger (1904), Echinomera hispida, Schellack (1907)—a granule of chromatin has been found expelled from the nucleus prior to gamete formation. Schellack (1907) believed that this must represent some sort of reduction process, especially in view of the fact that E. hispida had an odd number of chromosomes. Hoffmann (1909) found similar granules in Monocystis, but did not believe that they had anything to do with reduction. Brasil (1905) found that after conjugation the nucleus of Monocystis expelled some granules of chromatin into the cytoplasm, but thought they were of a vegetative nature. Thus Schellack's interpretation of the expulsion of the granules from the nucleus is not supported by any others, and, indeed, he himself does not seem to be greatly intrigued by it.

In 1904 Paehler described from Gregarina ovata a process which outwardly closely resembled the formation of polar bodies in the metazoan egg. Later Schnitzler (1905), who worked on the same species as Paehler, described this process still more fully. The gamete nuclei become surrounded by cytoplasm and break free from the parent organism. A centriole appears, which presumably divides into two, and a spindle is formed. "In a typical karyokinesis two daughter-plates are formed, of which one remains in the sporoblast and gradually rounds up to form the sporoblast nucleus, while the other becomes condensed and cut off as a reduction body." Only one division was said to take place, and no chromosomes were counted. From Schnitzler's figures it would seem as if three chromosomes were dividing into two groups of three; and although Schnitzler gives four as the chromosome number in the preceding nuclear divisions, his figures of these also frequently show three only. Later Léger and Duboscq (1909) describe a similar happening in Gregarina munieri, but here two "reduction" divisions take place, the "polar body" dividing into two. Again there is no evidence of a reduction in chromosome number, and although no chromosome counts are given, three chromosomes are shown in the figures. On the whole this seems to be a rather complex method of expelling a chromatin granule similar to those described in the previous paragraph. Schellack (1912) has re-examined G. ovata with great care, and he has found that the number of chrosomones is three, and not four as Schnitzler had said. Further, although he examined the necessary stages with the greatest care, in order to observe the "reduction" phenomena, he was unable to confirm the findings of the previous workers. He says, indeed, that the karyosome expelled a granule which made its way into the cytoplasm; but nothing more happened. He found no "polar body." This type of "reduction" has been found only in the genus Gregarina, and—which is noteworthy—only in species which have an odd number of chromosomes. It is difficult to understand how the three "somatic" chromosomes could be reduced (halved) in this way. Moreover, quite apart from the fact that no true reduction in chromosome numbers has been established, the phenomenon in question does not seem to be of constant occurrence. What the significance of the expulsion of a small portion of chromatin may be I cannot suggest; but I am inclined to believe that, like the previously described processes in Stylorhynchus and Echinomera, it has more to do with the vegetative than the reproductive side of the gamete's existence.

There remain two cases of reduction that are important because in them the chromosomes have been counted. They will have to be considered in detail. Mulsow (1911) described what he believed to be a reduction division in Monocystis rostrata, a new species which he found in the seminal vesicles of Lumbricus terrestris. This species was said to have eight chromosomes. There was only one reduction division, the last division before gamete formation. At this division eight chromosomes appeared as usual when the spireme became segmented. They were thought to be associated in pairs but not actually united, as, on analogy with a metazoan reduction, Mulsow expected. The eight chromosomes then separated into two groups of four, a daughter-group going to each pole of the spindle. It has already been pointed out (Dobell and Jameson, 1915) that Mulsow was probably dealing with two species of Monocystis—one with eight chromosomes, the other with four—and that what he observed was not an instance of reduction but one of confusion.

In the first place it must be noted that Hesse (1909) has recorded six species of Monocystid gregarines from Lumbricus terrestris, and that Mulsow's new species makes a seventh. Several of these are very common, and mixed infections are of regular occurrence. To expect to find always a pure infection would seem to be folly. Even when the vegetative forms are easily enough distinguished the cysts are usually extremely alike. Further, we have already seen that four is not an uncommon number of chromosomes in various species of Monocystis. Mulsow himself, it seems to me, has supplied evidence which points to a mixed infection. He found always a certain number of division figures which were quite unlike those of Monocystis rostrata. He has given samples of these in his figs. 54–57. His explanation of these is that they are due to bad fixation, but at all events figs. 54 and 56 seem to be excellently fixed, and I would suggest that the differences may be due rather to the fact that the different spindles really belong to different species of Monocystis. Even in the figures illustrating the normal life-cycle of Monocystis rostrata there are a number of suggestive discrepancies. Thus in figs. 2 and 20, which show the origin of the chromosomes, only four chromosomes are figured. Of course it is conceivable that four more might form later; but this would be extraordinary, and it is far more probable that four is the complete number. It may be noticed also that the chromosome pairs shown in fig. 46 are not really pairs at all, but eight distinct and different chromosomes. Furthermore, if the figures showing the prophases—figs. 38–46—are compared with those —figs. 48–53—which are supposed to show the actual reduction, it will be seen that the chromosomes shown in the prophase figures are quite different from those shown in the division stages. Finally, in the reduction division itself, figs. 48 to 50 show the four daughter-chromosomes moving to each pole of the spindle, but still connected together at their ends—not eight chromosomes separating into two groups of four, but four chromosomes apparently dividing into two daughter-plates of four each. Mulsow noted the connection of the daughter-groups at this stage, but again blamed the fixation. The connection together of chromosomes by their ends or by delicate chromatin strands is a well-known feature of dividing chromosomes, but I doubt if any amount of bad fixation would bring it about in fully individualized chromosomes which were merely separating into two lots.

The more one examines Mulsow's case of "reduction" the less is one satisfied with it. To me the evidence seems to point strongly to the confusion of a species having four chromosomes with one possessing eight. If Mulsow's account of this division is correct, I can but conclude that it is a special form suited only to those species whose "somatic" chromosome number is even.

The other case in which a reduction in the number of chromosomes is said to have been observed is that of Stenophora juli, described by Trégouboff (1914). The "reduction" is after the fashion described above for Gregarina, but "un peu spéciale." No reduction could be found during the formation of the microgametes on account of the unfavourable material; but when formed they were seen to contain "two chromatin bodies placed side by side—certainly the chromosomes." The macrogametes become free and are fertilized. Reduction takes place "either before copulation or during copulation, or even after the spermatozoid has already penetrated the egg." The egg nucleus, at first a collection of distinct granules, forms four chromosomes. A division now occurs in which two chromosomes, generally smaller than the other two, are expelled from the egg. A small granule which is present at this division is interpreted as a centrosome.

This case is far from satisfactory. In the first place there is no account of any nuclear divisions prior to this "reduction" division. Consequently we do not know what the "somatic" number of the chromosomes is. We are told that the dividing nuclei "are very small and do not give good figures." It is strange that after gamete formation, when the nuclei must be considerably smaller than in the preceding divisions, Trégouboff was able to follow the reduction division "easily enough in detail, the material being particularly favourable for this study." Trégouboff's figures are unfortunately only text-figures and do not show much detail. It is to be presumed, however, that they show all the necessary points. What do they actually show? Fig. *c* is said to show "réduction chromatique dans l'œuf avant la copulation"; it actually shows, near the periphery of the egg, four round grains arranged in a square with a fifth smaller one immediately above them. Figs. *d* and *e* are said to show "réduction chromatique dans l'œuf et la copulation"; they actually show the same four granules arranged in the same way with the fifth grain occupying a similar position, while on the outside of the egg is a small black mass, presumably the microgamete. Fig. *f* is said to show "réduction chromatique après la pénétration du spermatozoïde dans l'œuf"; it actually shows once more the four round granules in square formation near the periphery of the egg, with a small black body (possibly two granules badly differentiated) lying near them; the fifth granule is absent. Fig. *g* claims to be the "syncaryon"; again there are seen the granules in "fours," the "centrosome" being absent, with two granules much smaller in size lying on the outside of the egg at the pole opposite to the "syncaryon." Fig. *i*, representing "the first mitosis in the copula," shows at one pole of a spindle (?) four granules and at the other two granules, with a dark streak joining the two groups. Of this stage Trégouboff says: "La première division mitotique dans l'œuf fécondé donnera deux noyaux contenant chacun 4 chromosomes encore nettement distincts à ce stade, et situés aux deux pôles du sporocyste (fig. *i*); quoiqu'on ne puisse l'étudier en détail, elle paraît être du type à un long chromosome axial."

I cannot think that any evidence is here shown to prove that there is a reduction division at all. There is no trace of a division figure unless the single "centrosome" is doing duty for it. There is no sign of the "expulsion of two of the chromosomes" except that in fig. *g* the two granules on the periphery are said to be these. There is no proof that four chromosomes are present in any stage of the life-history. To establish this reduction a full series of figures showing all phases in the divisions is necessary, and until these are published it is almost impossible to determine what we are dealing with in this case. It seems to me that Trégouboff has over-estimated the ease with which he could follow the details of the reduction division. I should be inclined to class these stages with the earlier divisions—"Elles sont très petites et ne donnent pas de belles images." In the absence of any evidence showing what the somatic number of chromosomes is, I suggest that it may possibly be two, and that what Trégouboff has taken for a reduction is merely an expulsion of a portion of chromatin such as has been already described in the genus Gregarina, the four globular chromosomes arranged in square being two daughter-plates of two chromosomes each. Again I must add that, even supposing Trégouboff's account is correct, it can only apply to those gregarines whose chromosome number is even; for those with an odd chromosome number we have still to seek a method of reduction.

Other reduction processes have been discussed by some authors, but as they are of a purely speculative character, without the support of any evidence whatsoever, they seem to me to be unworthy of consideration.

It will be clear, I think, from the foregoing considerations that the search for reduction divisions—in the proper sense of the term—in the gregarines generally has hitherto been vain. The so-called "reductions" by extrusion of chromatin granules have clearly nothing to do with the true chromosome reduction (meiosis) of other animals and plants. The attempts to discover a meiotic phase in the nuclear divisions preceding or accompanying gametogenesis have also failed. Such stages as have been found—and they are neither many nor serially complete—have been arbitrarily interpreted in such a manner as to make them fit into a general scheme of reduction and gametogenesis derived from, and therefore appropriate to, a metazoan animal. Reduction has been sought at a stage when it ought to occur according to certain preconceived notions. It has not been found, because in all probability it does not occur at this stage. The true reduction of the chromosomes occurs at a stage where it has hitherto not been sought; consequently no amount of hypothesis and argument over details has been able to surmount or explain the fundamental mistake which has been made in all the earlier attempts at interpretation.

F. The Gametes.

The forms presented by the gametes of gregarines are very varied. It would be fruitless to go over the ground that has been so carefully covered by many authors, especially by Léger and Duboscq, and the more so because the gamete nuclei—with which I am here chiefly concerned—are in many cases very much alike although the general appearances of the gametes may be so different. I shall only recall the fact that those who have studied this point seem to be of the opinion that the gametes are nearly always anisogamous. Even when isogamy has been described by one worker, more ingenious later observers have often been able to demonstrate some minute differences between the "male" and the "female" gametes. Hoffmann (1909) has pointed out that anisogamy is often more marked in the living gamete than in the fixed and stained preparation. I confess I have not concerned myself very much with this question in Diplocystis schneideri. To me the gametes appear to be exactly alike, and I can distinguish no structural differences between the members of a pair, though it is possible that minute differences might be discoverable if more diligent search were made.

In this connection it may be recalled that the two gametes of Diplocystis, though to all appearance structurally identical, may possibly be slightly different in their functional characters. It has already been pointed out that the zygotes tend to be found towards one end of the cyst, and this would seem to indicate that the gametes formed by one of the associated individuals are more actively motile than those of the other, and migrate from one end of the cyst to seek their partners at the other end. It might therefore be justifiable to regard these two classes of gametes as "microgametes" and "macrogametes" respectively, and the organisms which form them as respectively male and female; and such a conception would, perhaps, enable us to reconcile the apparently complete structural isogamy of Diplocystis with the condition of extreme anisogamy which obtains in so many other gregarines.

One other point is also worthy of note here. In Diplocystis all the gametes formed by both individuals of an associated pair are, as we have seen, exactly alike as regards their chromosomes. Neither the "male" nor the "female" individual forms gametes distinguishable by their chromosomes into two categories. So far as can be judged at present, from the incomplete publications of others, this is also true of many other gregarines. There is no "accessory" or "sex" chromosome in these organisms, and the "unpaired" or "axial" chromosome which has been described is clearly not the homologue of the "sex chromosome" of an insect or other animal displaying a chromosomic dimorphism in its gametes. The "unpaired" chromosome of a gregarine is, indeed, unpaired: but so are all the other chromosomes which accompany it; for the nuclei at all stages, from the first division in the gamont up to the last division before gametogenesis, contain a single set of chromosomes—a haploid and not a diploid group. This fact, if, as I believe, true of all gregarines—including those with conspicuous sexual differentiation—should be of interest to those who regard "accessory" chromosomes as "sex determinants" in the Metazoa.

G. The Sporoblast (Zygote) and its Nucleus; the True Reduction Division.

The behaviour of the nucleus in the sporoblast and its division into the eight sporozoite nuclei have never been carefully examined previously. As a rule it has been sufficient for the investigator to mention that the divisions took place—some describing a "primitive division," others ordinary mitosis. In Diplocystis schneideri for the first time the nuclear divisions within the sporocyst have been carefully examined; and, as we have seen, the results are interesting and perhaps of far-reaching importance. To recapitulate them briefly: The sporoblast is a zygote. Its nucleus is formed by the fusion of the two gamete nuclei, each containing a similar set of three chromosomes. The first nuclear division of the sporoblast is a reduction division. From the spireme, which is formed during the early prophases, six chromosomes arise. These lie on an indistinct achromatic spindle and separate into two homologous groups of three, one set of three passing to each pole. A second and a third mitotic division then take place—each presenting the same number (three) of chromosomes—giving rise, finally, to the eight sporozoite nuclei. The sporozoites thus each contain three chromosomes—a single or haploid group. This is the number present again in all the nuclear divisions—including all the divisions associated with gametogenesis—of the gamont, which is the adult organism into which the sporozoite grows.

This type of reduction division is new, and it explains in a simple fashion the odd chromosome numbers so common among gregarines. The number found in every division excepting the first sporoblast division is the haploid number. Only once in the whole life-cycle is the diploid number found, namely, in the first division in the sporoblast. It seems probable that a careful re-examination of gregarine life-histories, paying special attention to the sporal divisions, will reveal this to be the real method of reduction in all.

Although Diplocystis is the only gregarine in which this type of reduction division has been found, it does not stand alone among the Sporozoa, for Dobell (Dobell and Jameson, 1915) has recorded an exactly similar phenomenon in Aggregata eberthi. Here the haploid number of chromosomes (six) occurs all through the schizogonic cycle of the parasite in the crab, and in every nuclear division in the male and female individuals in the cuttle-fish. At the first division in the zygote, however, the diploid number (twelve) occurs, consisting of two homologous groups of six differentiated chromosomes, derived from the macrogamete and microgamete nuclei. At no other stage in the life-cycle is the diploid number present. Reduction, as in Diplocystis, takes place during the first division of the zygote nucleus, the chromosome number here being halved from twelve to six. The chromosome cycle of Aggregata is thus closely comparable with that of Diplocystis, though it must be remembered that the stage in the life-history at which fertilization occurs is not the same in Coccidia and Gregarines, the zygote being a sporont in the former, a sporoblast in the latter.

Conclusions.

Of the several interesting questions which the foregoing review has touched there are only two on which I wish to lay special emphasis :

(1) The peculiar origin of the karyosome in Diplocystis schneideri, in which a "micronucleus" makes its way inside a nucleolus, giving rise to a "karyosome" composed of

two clearly differentiated portions, taken in conjunction with the fact that in many other Gregarines the karyosome is similarly constructed, opens, I think, a very important question. The entire gregarine "nucleus" is clearly not exactly comparable with the nucleus of a metazoan cell. It is probably a much more complex organ, comparable with a nucleus within a nucleus. Such evidence as I have been able to bring forward is insufficient to exhaust this question, which is undoubtedly one that would repay further inquiry.

(2) Heretofore, reduction in gregarines has been sought for in the two nuclear divisions immediately preceding gamete formation. I have tried to show that in none of the so-called "reduction divisions" which have been described has a true reduction been demonstrated. In Diplocystis schneideri the reduction division has been found to be the first division in the sporoblast, and this is probably the stage at which it occurs in other forms. This mode of reduction offers a simple explanation of the odd chromosome number which is so common in gregarines; it is the haploid number, which is present in every nucleus in the whole life-cycle except the zygote nucleus.

Finally I would like to draw attention to the fact that both of the above important points emphasize the difference between the Protozoa and the Metazoa. In the present case, especially as regards reduction, a somewhat slavish following of metazoan embryology has, indeed, already obscured the truth and led into serious error. Dobell (1911) has shown the futility of interpreting the Protozoa in terms of the metazoan cell, and yet it still goes on. Clarity of thinking will not come in Protozoology until the Protozoa are fully recognized as non-cellular organisms, comparable with whole metazoan individuals rather than with their single component cells.

Imperial College of Science and Technology;
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EXPLANATION OF PLATES 12 TO 15.

Illustrating Mr. A. Pringle Jameson's paper on "The Chromosome Cycle of Gregarines, with Special Reference to Diplocystis schneideri Kunstler."

[The figures were all drawn with the aid of a camera lucida. Excepting for figs. 1, 25, 26 and 27 a Leitz 2 mm. apochromatic lens with compensating oculars was used. Unless stated otherwise the figures are from sections stained with Dobell's hæmatein stain and light green.]

PLATE 12.

Fig. 1.—A Diplocystis "pair" from the body-cavity of a cockroach, showing in each a large nucleus with several karyosomes. Whole mount. Borax carmine. × 65.

Fig. 2.—Spore showing sporozoites coiled within. Smear from ripe cyst. Acid carmine. × 1600.

Fig. 3.—Spore showing line of dehiscence. Fresh. × 1600.

Fig. 4.—Empty sporocyst showing triangular opening and sporozoite lying beside it. Smear from gut of cockroach fed on spores. × 1600.

Fig. 5.—Sporozoite showing nucleus. It has become rather flattened in the preparation. Smear. × 2500.

Fig. 6.—Spore commencing to dehisce. × 2500.

Fig. 7.—Sporozoite in gut-wall. Smear. × 1600.

Fig. 8.—Sporozoite in gut-wall. Growth has commenced. The oval netted bodies are the nuclei of the cells. × 1600.

Fig. 9.—Sporozoite in gut-wall. Later stage showing amœboid appearance. × 1600.

Fig. 10.—Section of gut-wall showing four parasites, the two older ones beginning to associate. × 1400.

Fig. 11.—Enlarged view of sporozoite in fig. 8, showing first stage in formation of the karyosome; the anterior granule enlarged. × 2500.

Figs. 12–19.—Young parasites showing stages in the formation of the karyosome; fig. 12 shows the two granules in contact; figs. 13–15 show the small micronucleus penetrating into the nucleolus; fig. 16 shows a solid micronucleus within the nucleolus; figs. 17–19 show the micronucleus becoming vacuolated. × 2500.

Figs. 20–25.—The disintegration of the karyosome in older parasites. Only the nucleus is figured. Fig. 22 shows an unusual method of disintegration, the micronucleus being extremely vacuolated and giving off dark masses into the nucleoplasm. Figs. 20–24 × 1600, fig. 25 × 850.

Fig. 26.—Still later stage in the disintegration of the karyosome, showing food-granules beginning to form round the nucleus. × 700.

PLATE 13.

Fig. 27.—Portion of gut-wall showing numerous parasites lying in the thickened basal layer; the gut is somewhat diagrammatic. × 100.

Fig. 28.—Portion of gut-wall showing a pair of very large parasites in it. Reconstructed from two sections. Borax carmine. × 100.

Fig. 29.—Very young parasites in sporulating Diplocystis cyst. The numerous black ticks are spores. Reconstructed from twelve sections. Heidenhain's iron-alum hæmatoxylin and Bordeaux red. × 100.

Fig. 30.—Junction line of two young associates, showing fusion at point of contact. × 900.

Fig. 31.—Junction line of two old associates, showing thinning at point of fusion, the delicate inner membranes, the alveolar wall, and the delicate outer membrane surrounding the two parasites. × 1000.

Figs. 32 and 33.—Nuclei of large parasites, showing the breaking down of the karyosome fragments to chromatin particles. × 450.

Fig. 34.—Nucleus of parasite about to commence prophases of first division. Note the three rather delicate and regular vesicles. Reconstructed from six sections. × 700.

Fig. 35.—Nucleus in early prophase of first division, showing several tiny vesicular fragments of karyosome and a single aster. Reconstructed from seven sections. × 700. See footnote, p. 222.

Fig. 36.—The aster in the previous figure highly magnified, showing the break in the nuclear wall. × 2500.

Fig. 37.—Karyosome vesicle from nucleus in early prophase, showing budding at one end. × 900.

Fig. 38.—Two asters lying outside the nucleus among the food-granules, four of which are shown, but surrounded by extruded nuclear material. A small karyosome vesicle is seen within the nucleus. Safranin and light green. × 1400.

Fig. 39.—Nucleus in early prophase of first division, showing aster at periphery where the nuclear wall has broken down. The karyosome vesicle is seen in optical section, showing the thickened hemisphere. The aster is really dividing into two at right angles to the plane of the paper, so that it cannot be shown in the figure. The nucleus is from one member of a pair, a part of the nucleus of the other member having been figured in the previous figure. Safranin and light green. × 900.

Fig. 40.—Early prophase of first division, showing a single aster some little distance within the nucleus and a karyosome vesicle lying beside it. × 1400.

Fig. 41.—Origin of the chromosomes of the first division. At the top of the figure is an aster with a small karyosome vesicle lying beside it from which the chromosomes are coming off. At the bottom of the figure is seen a large karyosome vesicle. Reconstructed from two sections, the aster and chromosomes being in one section and the large karyosome vesicle in the other. × 900.

PLATE 14.

Fig. 42.—Highly magnified view of the origin of the chromosomes figured in fig. 41. There are seen to be three karyosome vesicles, two of which are very tiny. They are partly overlying the aster. Coming off from them are three filamentar chromosomes, two of which have thickened ends. × 2500.

Fig. 43.—Metaphase of first division. The chromosomes have condensed to small globular bodies and are dividing. Two fragments of karyosome are lying partly on the figure. Division is taking place in a homogeneous ground of nuclear débris. × 2500.

Fig. 44.—Late anaphase of first division, showing a vacuole forming round each daughter-group of chromosomes. × 2500.

Fig. 45.—Late telophase of first division. The daughter-nuleci have drawn far apart and are almost completely reconstructed. The asters are still large. The nuclei are lying among food-granules. × 1400.

Fig. 46.—Completely reconstructed nucleus from first division. The aster is shrunken and solid looking. × 2500.

Figs. 47 and 48.—Second division figures. Note the web-like appearance of the protoplasm and the aster rays. The chromosomes are small and globular. × 2500.

Figs. 49–51.—Third division figures. Note that the chromosomes in the early phases are filamentar, in the later short and stumpy.

Figs. 52–63.—Fourth and fifth division figures. × 2500.

Figs. 52–55.—Division of aster into two, disintegration of karyosome and formation of spireme.

Fig. 56.—Formation of three chromosomes from spireme.

Fig. 57.—Formation of spindle from aster rays invading the nucleus.

Fig. 58.—Chromosomes on the equatorial plate.

Fig. 59.—Spindle viewed from one pole, showing the chromosomes splitting.

Figs. 60–62.—Anaphases.

Fig. 63.—Early telophases.

PLATE 15.

Fig. 64.—Late telophase of fifth division. × 2500.

Fig. 65.—Reconstructed nucleus from fifth division. × 2500.

Figs. 66–74.—Nuclei dividing at the periphery. × 2500.

Fig. 66.—First division at the periphery, showing asters.

Figs. 67 and 68.—Later peripheral division, showing cones projecting from surface.

Figs. 69 and 70.—Still later peripheral divisions, showing nuclei projecting from the surface and division taking place almost entirely within the nuclear membrane. The chromosomes are much condensed.

Figs. 71–74.—Last division at the periphery. Three chromosomes can still be clearly seen.

Fig. 75.—Portion of periphery of parasite, showing layer of cytoplasm. × 100.

Fig. 76.—Nuclei at periphery just about to form gametes. × 1600.

Fig. 77.—Gametes forming and nearly completely formed. The partition between the two individuals in the cyst has broken down. × 1400.

Fig. 78.—Fully-formed gamete. × 2500.

Figs. 79–83.—Conjugation. × 2500.

Fig. 79.—Two gametes united but not yet rounded up.

Fig. 80.—Gametes united and rounded up.

Fig. 81.—Complete fusion of cytoplasm; nuclei not yet united.

Fig. 82.—Nuclei united but chromatin masses separate.

Fig. 83.—Completely formed zygote.

Figs. 84–111.—Nuclear divisions in the sporoblast and formation of spore. × 2500.

Fig. 84.—Karyosome condensed.

Fig. 85.—Karyosome breaking up to form spireme.

Figs. 86–88.—Formation of spireme.

Figs. 89–92.—Synapsis.

Figs. 93–95.—Formation of six chromosomes from the spireme after synapsis. Figs. 93 and 94 are the more typical, but in fig. 95 the six chromosomes are more easily counted.

Figs. 96 and 97.—Separation of the six chromosomes into two groups of three—the reduction division.

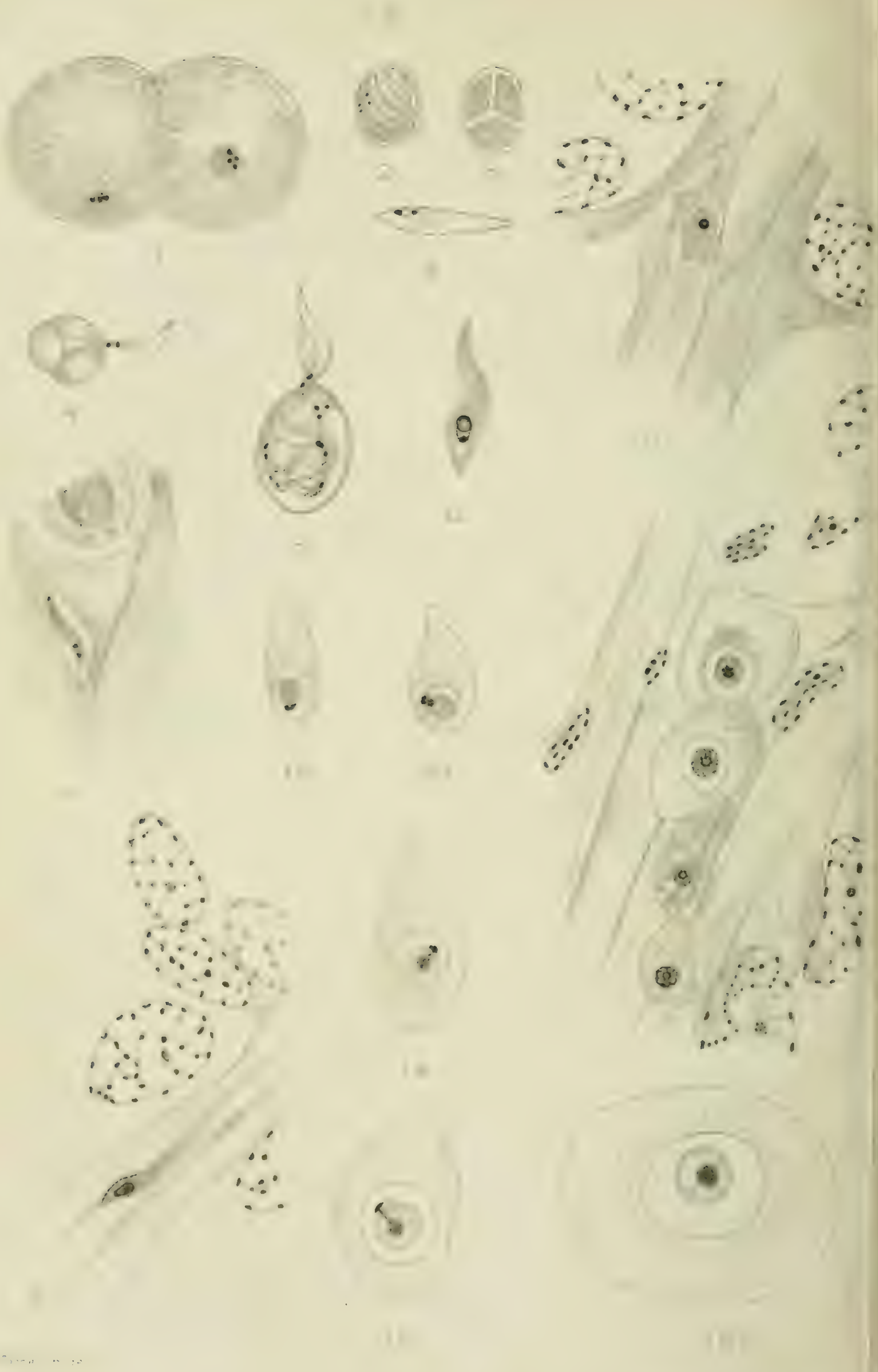
Figs. 98 and 99.—Reconstruction of the two daughter-nuclei.

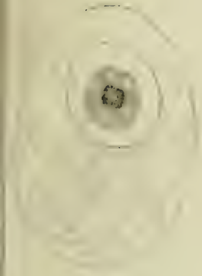
Figs. 100–104.—Division of the two nuclei into four.

Figs. 105–108.—Division of the four nuclei into eight.

Fig. 109.—Spore with eight nuclei.

Figs. 110 and 111.—Two "chips," containing four nuclei each, of spores at eight-nucleate stage, showing the chromatin becoming condensed into two caps.





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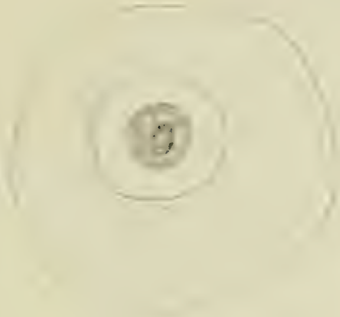

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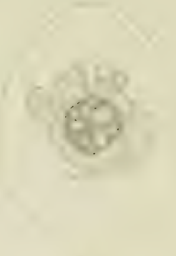

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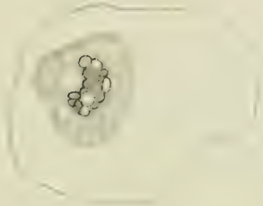

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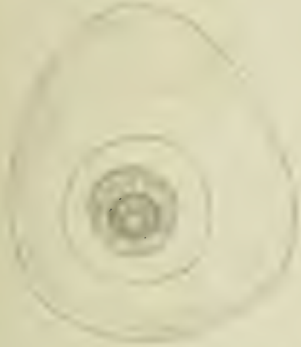

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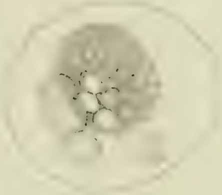

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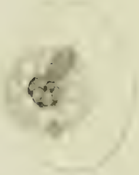

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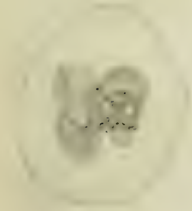

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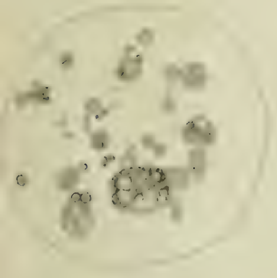

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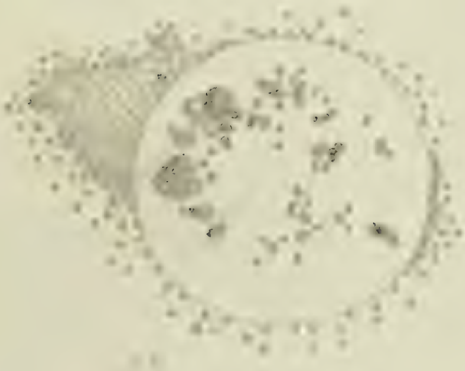

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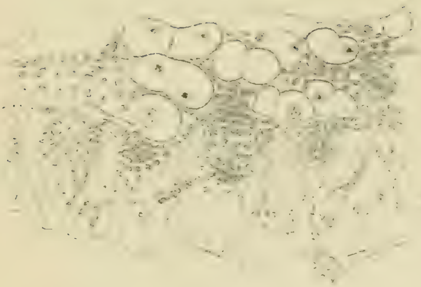
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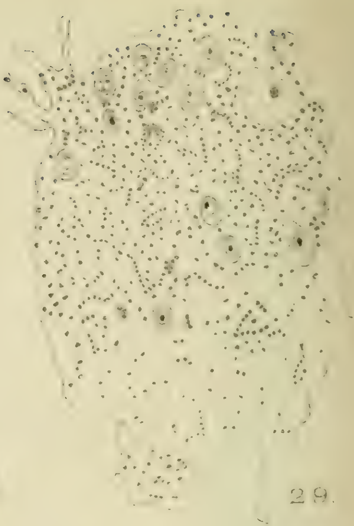
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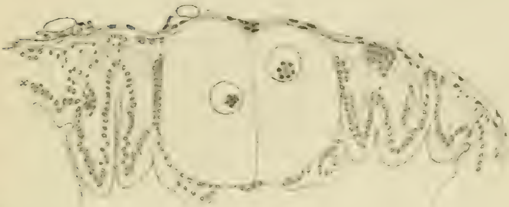
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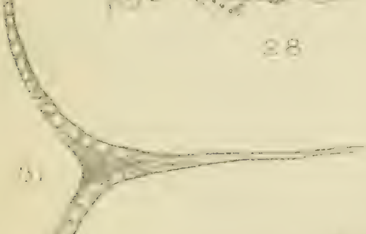
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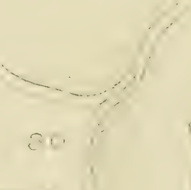
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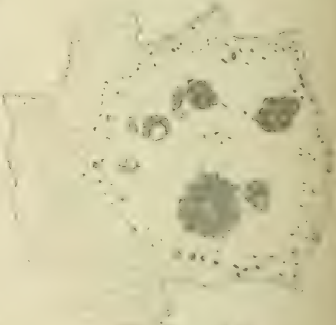
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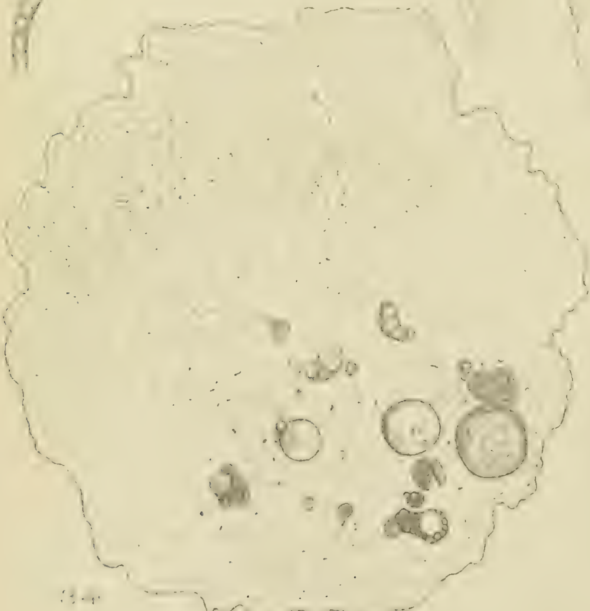
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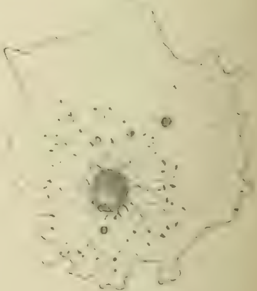
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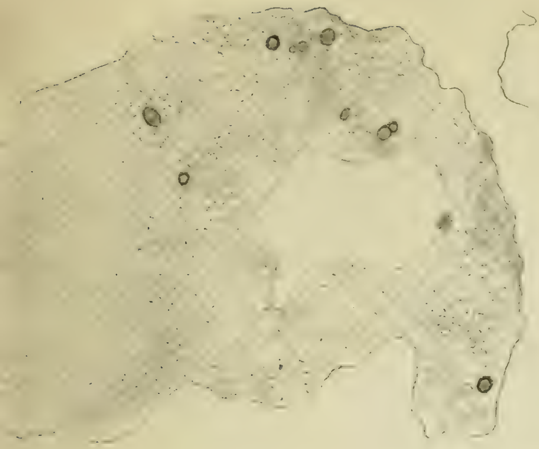
CHROMOSOMES



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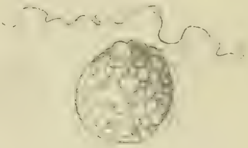

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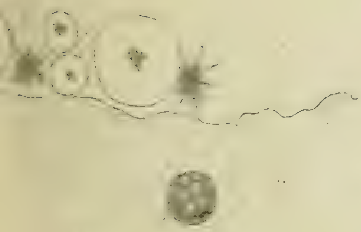

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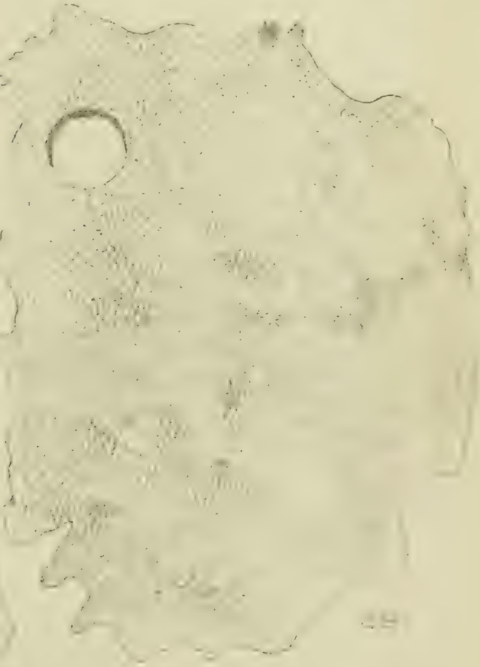

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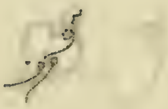

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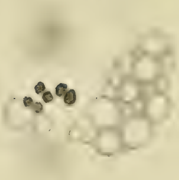

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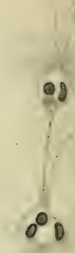

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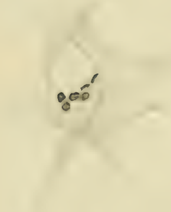

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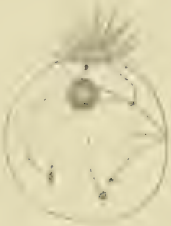

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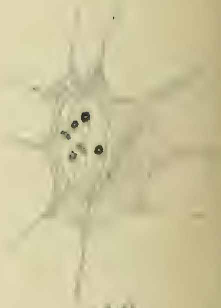

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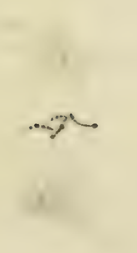

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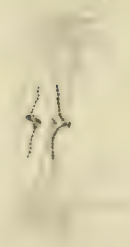

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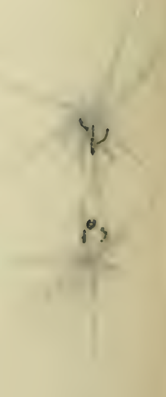

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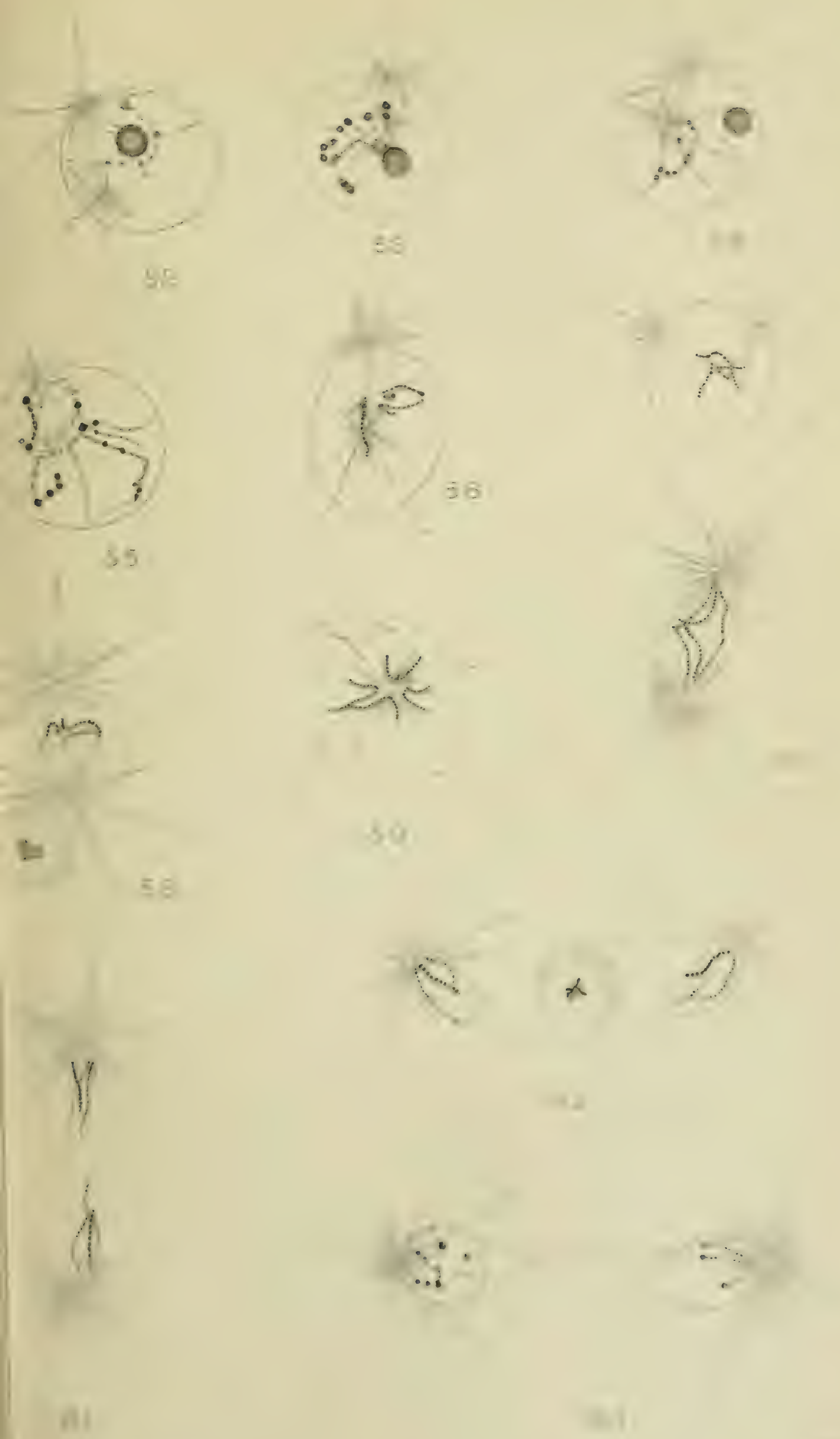

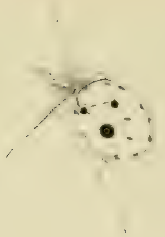

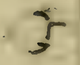

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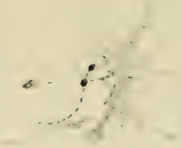

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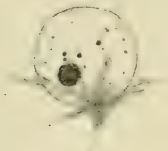

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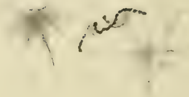

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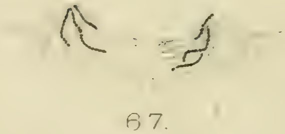

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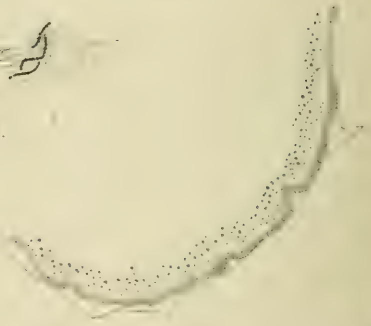

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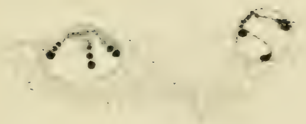

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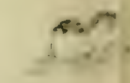

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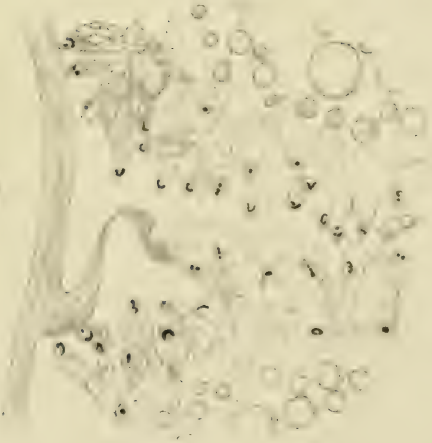

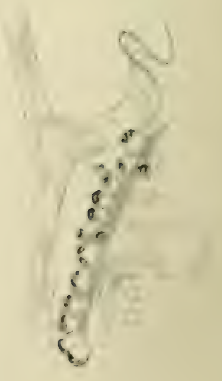

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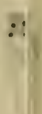

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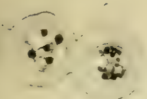
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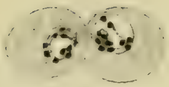
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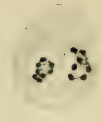
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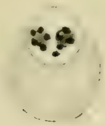
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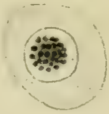
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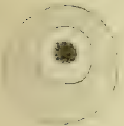
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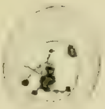
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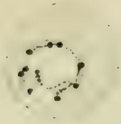
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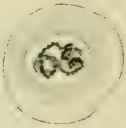
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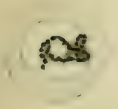
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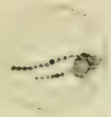
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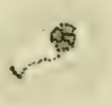
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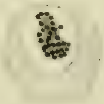
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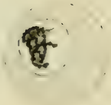
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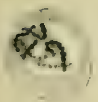
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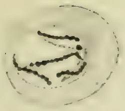
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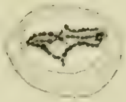
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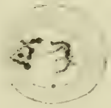
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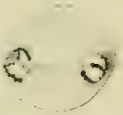
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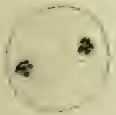
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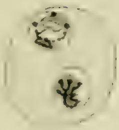
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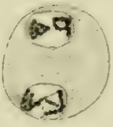
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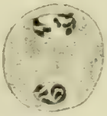
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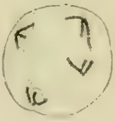
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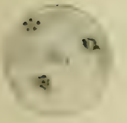
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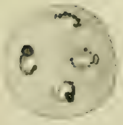
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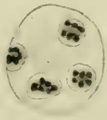
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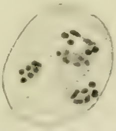
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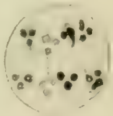
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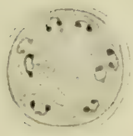
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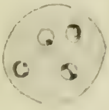
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OF GREGARINES.

The Cytoplasmic Inclusions of the Germ-Cells.

PART VII. THE MODERN TECHNIQUE OF CYTOLOGY.[1]

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1. INTRODUCTION.

In this paper I have collected some notes on technique, with especial attention to various methods used for demonstrating and distinguishing between protoplasmic and deutoplasmic bodies. I have also added directions and a plan for research on the lines intoduced into this country by myself.

In a previous paper published in the 'Journal of the Royal Microscopical Society' (**13**) I have gone into certain other aspects of these questions, and this paper is meant to supplement what I have written before. The application of modern technique to experimental zoology and physiology will, I have little doubt, very shortly provide us with a wealth of facts with regard to the life of the cell and the development of the organism.

Where necessary I have made due acknowledgment to other workers, such as Murray, Cramer, Cowdry, Bensley, Kull, Hirschler, Regaud, Champy, Cajal, Golgi, etc.

Formulæ of fixatives such as Bouin, Flemming, Altmann, Champy, Gilson-Petrunkewitsch, Carnoy, etc., will be found in books on microscopical technique, and are not given here. This paper is written with the supposition that the reader is in possession of a late edition of Bolles Lee (**3**) or some other such admirable book. Complete directions for every new method in this paper are given carefully, and full bibliographies will be found in the other parts of this series of papers.

2. The Cell Elements Classified.

In the scheme on p. 270 I have given a tentative classification of the cell elements. The nucleus contains four well-known categories of substances or materials, and in addition in some cells it can be shown (Rio Hortega, **29**) that an enigmatic granule or rod which is argentophile is seen to lie in or near the nucleolus. I have called this rod the "Rio-Hortega Body" after its discoverer, who is one of Cajal's pupils.

Between the words nucleus and cytoplasm I have drawn a double arrow to denote the interaction which goes on between these bodies. The cytoplasm is known to contain two general categories of bodies—metaplasmic or dead storage, excretion or secretion products, which also fall under the term "deutoplasmic," especially if they are nutrient, and protoplasmic bodies (active centres of vital changes), engaged in processes of cell metabolism. Between these two groups I have drawn a double arrow to denote exchange.

The deutoplasmic inclusions are especially fat, yolk, glycogen and "lipoid" vacuoles, while excretion granules and pigment are possibly different and are truly metaplasmic, but for the present it is somewhat difficult to separate out the dead cell inclusions into distinct classes. Pigment for instance may be associated with mitochondria and possibly also with Golgi elements.

With reference to the word "dead" and "living" as used in this classification, it should be pointed out that both mitochondria and Golgi elements are centres of cytoplasmic activity, and can be seen to grow and divide. Yolk-discs and fat-droplets are classified as dead, because they do not divide, and are not centres of cytoplasmic activity in the same sense or way as the mitochondria or Golgi elements.

Classification of Cell Elements.

- A. **Nucleus** with nuclear inclusions.
  1. Chromatin (chromosomes).
  2. Nucleoli
     - karyosome.
     - Plasmosome.
  3. Linin.
  4. Karyolymph (nuclear sap).
  5. (Rio-Hortega body ?)
- B. **Cytoplasm** with cytoplasmic inclusions.
  - α. Protoplasmic (cytoplasmic) or living inclusions.
    1. Golgi apparatus (sometimes with archoplasm or sphere).
    2. Mitochondria.
    3. Isolated chromatoid body, neutral red granule, micromitosome, etc.
  - β. Deutoplasmic (metaplasmic) or dead inclusions.
    4. Fat.
    5. Lipins other than lecithin.
    6. Yolk (proteid, lecithin and fat).
    7. Glycogen (animal starch).
    8. Pigment-granules.
    9. Zymogen and excretion granules
  - γ. Ground cytoplasm (protoplasm).
  - δ. Centrosome (sometimes with archoplasm or sphere.)
  - ε. Chromidia, secondary nuclei (bodies of nuclear origin and chromatinic nature, rare in Metazoa).

It is certain now that both protoplasmic and deutoplasmic inclusions are closely inter-related. Murray (**24**) and Dubreil (**11**) both describe the metamorphosis of mitochondria (living) into fat-globules (dead). It is also true that mitochondrial, zymogen, pigment and lipin granules or vacuoles are closely connected with one another. This serves to warn us that such a classificatory scheme as I have given here must not and cannot be regarded as rigid. That such a classification is useful I believe that there is no doubt, and it gives one some idea of the complexity of the cell.

Under the section of Cytoplasm I have given the ground protoplasm or cytoplasm and the centrosome a special heading by themselves, as is justified by their importance.

A classification of this sort can be the only basis of the cellular side of research in embryology, because we now know that the nucleus and ground cytoplasm are only two of the many bodies and substances which form the cell. The development and life of the organism are comprised by the behaviour and changes which go on in the complex system outlined in my classification. Attention is drawn to the tables which I have published elsewhere, and which are aimed at enabling the observer to identify any special cell-body by its fixing and staining reactions (**13**).

3. Are the Mitochondria and Golgi Elements Artefacts Produced by Certain Techniques?

Within the last few years the work of Regaud, Champy, Fauré-Fremiet, Meves, Held, Hirschler, Weigl, Nusbaum-Hilarowitz and Cowdry abroad, and of Murray and myself in this country, has focussed a good deal of attention on the mitochondria. More recently also I have entered into the question of the Golgi apparatus in zoological material, and Weigl, Cajal and Hirschler, too, have written papers on the Golgi apparatus in eggs. Naturally this attention has also been partly constituted by a scepticism as to the facts which I have lately published under the title of "The Cytoplasmic

Inclusions of the Germ-cells." The sceptics are almost always found to be ignorant workers who are amateur cytologists and microscopists, interested mainly in diatoms, and the mechanism and theory of microscopes. To these I commend the following facts, which even a cursory study of the international literature will confirm.

(1) The mitochondria can be seen and studied under the microscope, in freshly teased live animal-cells, or in the hairlets of the roots of plants, etc. (**12**, **15**, **20**).

(2) The Golgi apparatus of many sperm-cells is quite easily seen under the same circumstances.

(3) Intra-vitam stains tinge the same bodies, in the same location, in live untreated cells.

(4) Properly fixed and stained cells show the same bodies, the same size and shape, in the same location as in paragraphs (1), (2) and (3).

(5) Fixing and staining methods reveal such bodies in all stages of the germ-cell cycle in a variety of animals and plant-cells.

(6) The presence of a Golgi apparatus and mitochondria in animal-cells is now widely accepted by trained workers.

4. Lipoids, Lipins, Phosphatides, Phospholipins or Substances in the Cell Allied to Fats.

Fats are soluble in acetone, xylol, chloroform and ether especially. It is found, however, that strong alcohol is not without effect on animal fat-globules. Now besides the true neutral fats, such as palmitin, stearin, and the unsaturated olein, it has been ascertained that there are other "fatty" substances in all cells, which are somewhat allied to the fats in their chemical nature. Such substances, which are broadly like the fats in their solubility reactions, have been called "lipoids," and are especially soluble in alcohol. Now it has been shown by the excellent worker Maclean (**21**), that ordinary fat solvents such as ether and chloroform, and also alcohol, extract a variety of different "lipoids" from the cell, ranging from cholesterol to pigments, and that while such substances agree generally in their solubility reactions, they may be otherwise unrelated. Leathes (19), in 1910, suggested that the word "lipin" be used for Thudichum's so-called "amidolipotids," which may only be decomposition products. In order to clear up the confusion with regard to these peculiar "lipoid" substances, Maclean has suggested that the term "lipin" be used only for those substances of a fat-like nature, which contain in their molecule either nitrogen or nitrogen and phosphorus, and which yield on hydrolysis fatty acids or derivatives of fatty acids.

In the following section I intend to use the word "lipoid" in Overton's sense, i.e. to mean all those substances which dissolve in organic solvents, such as chloroform, alcohol, and ether.

The lipins comprise two special groups of substances—the phosphatides and the cerebrosides; the term "phospholipin" has been suggested instead of phosphatide. Phosphatides are fat-like bodies containing in their molecule nitrogen and phosphorus, while the cerebrosides contain nitrogen but no phosphorus. Acetone will not dissolve lipins but will dissolve fats. Biochemists extract lipins from dried tissues by means of ether and alcohol and precipitate the bodies from the solvent by means of acetone. Certain "lipins" at least form insoluble compounds with chrome mercury and platinum salts. "Lipoids" (e.g. cholesterol, lecithin, fat, cerebrin, etc.) form a special part of almost all cell-organs, as seems to be indicated by fixing tests, and so far as we know such substances are always intimately associated with protoplasm. From this we arrive at a definite conclusion as to the importance of work on "lipoids"; it is this: fixatives containing alcohol, chloroform and acetic acid especially either completely dissolve away "lipoids" of all kinds, or distort and alter their morphological arrangement in the cell or cell organs; while those fixatives which in themselves are not injurious, but which do not cause the lipoids to become insoluble in the dehydrating and clearing reagents, are not indicated for the best cytological work.

5. Yolk-Discs and Spheres.

Nearly all, if not all eggs contain yolk-bodies of some sort. In the majority of cases such yolk-bodies are spherical or discoidal, and are constituted by lecithin, free fat, and a coagulable protein which has been called vitellin (**27**). According to the amount of each of these three categories of substances, the yolk-sphere or disc will act in a varying manner under a given technique. In osmic acid the sphere may or may not go black, and I think this depends on the presence or absence of unsaturated free fat; in other cases it may or may not stain black or violet in iron-hæmatoxylin or Benda (**3**) respectively, and this apparently depends on the quantity of the protein contained in the disc.

Yolk-discs or spheres form most of the nutrient substance in eggs, and no two different animals have yolk-granules of the same chemical constitution. In birds the yolk-discs go black almost immediately in osmic acid, while in the frog and snail they do not do so. I do not think the yolk-disc of the frog is phyletically a different structure from that of a bird or a snail, and probably the yolk-disc of any animal will be found to contain the three categories of substances—fat, lecithin, and proteid—in stable equilibrium. Since true yolk-spheres contain either lecithin or proteid it is not difficult to discriminate between them and fat-spheres. Fat-spheres or vacuoles, yolk-spheres, and true lipin spheres, such as occur occasionally in fatty tissue and liver, are nevertheless liable to become confused with one another. (See section on turpentine extraction, p. 287.)

6. The Problem of Fixation.

Older cytologists were satisfied if their preparations showed the nuclei and cell walls unshrunken and clearly marked. Nowadays, however, it can be recognised that the problem of fixation is complicated. Besides the coagulable proteins, the cell contains a number of substances, many of which are so easily altered or dissolved that special precautions must be observed in fixation. The materials in the cell which collectively fall under the term "lipoid" are rapidly destroyed by alcohol, acetic acid, chloroform, and are altered by certain other fluids. Not only this, but it is necessary to mix one's reagents in such a way as to avoid distortion; it should be noted that the problem of fixation is not constituted only by the questions as to how various chemical reagents affect any cell substance, but also by the question as to how such a treated substance will resist the subsequent dehydration and embedding. For instance formol fixation does not injure fat in cells, but it will not keep alcohol and xylol or chloroform from subsequently dissolving it out of the tissue; osmium tetroxide also does not injure fat in cells, but in addition it keeps the alcohol and xylol or chloroform from dissolving it away subsequently.

From the cytological and protozoological points of view the problem of fixation may be said to be constituted by two difficulties: (A) Avoidance of fluids which dissolve out or distort cell elements; (B) avoidance of fluids which enable or cause cell materials to be soluble in the dehydrating and clearing agents. As an example I will take the technique widely used by protozoologists, i.e. that of Schaudinn's fluid or Bouin's fluid and iron-alum hæmatoxylin. Schaudinn's fluid is a typical alcohol-acetic acid and corrosive sublimate fixative; the application of it to the cell possibly results as follows: Some of the proteids are coagulated, but part at least of such coagulated proteids are water-soluble. The acetic acid and alcohol rake all lipins out of the cell, while other fatty bodies are either dissolved away, or left in a state which allows them to be dissolved away in subsequent dehydration and clearing. Then the cells are stained in watery alum hæmatoxylin, which dissolves out those water-soluble proteid salts. Some workers have partly done away with the latter difficulty by using an alcoholic hæmatoxylin. Bouin's fluid is possibly less actively destructive than Schaudinn's but it is not indicated for cytological protistology. A paper bearing on this subject has recently been published by Dr. Ward Cutler, who has especially studied Ditrichomonas, a remarkable flagellate from the gut of a termite (**10**). In certain Trichomonads are found a row of coarse protoplasmic granules, whose correct identity is difficult to recognise. Cutler calls them "metabolic granules," and he shows that in most ways chrome osmium fixation gives the truest picture of the fixed organism. There is no other branch of zoology quite like protistology, for in the latter the most perfect technique is needed and is rarely found. Most protozoologists publish figures of their bad preparations, with the mistaken opinion that what they draw is something like the organism intra-vitam.

7. Ten Common Reagents Used as Fixatives.

These reagents are as follows:

- A.
  1. Osmium tetroxide in water.
  2. Formaldehyde gas in water.
  3. Chromium trioxide in water.
  4. Bichromate of potassium in water.
  5. Platinum chloride in water.
- B.
  6. Mercury bichloride in water.
  7. Nitric acid.
  8. Picric acid.
  9. Alcohol.
  10. Acetic acid.

Chloroform and urea are also used. In the group marked A are arranged the valuable reagents, in B the less valuable or destructive ones. Good fixatives can be made from the substances in group A without using any of the reagents in group B. The latter contains the reagents useful for chromosome work, the former reagents useful for fixing the cytoplasm and "resting" nucleus.

From group A have been made the following mixtures: Altmann, Champy, and Flemming and Hermann-without-acetic acid; these are among the best mixtures known. Then there are formalin (5 per cent. to 10 per cent.), Regaud, formol-Müller and formol-Flemming, which are so good for mammals. Good general micro-anatomical fixatives from group B are Zenker, Bouin, Gilson-Petrunkewitsch and Schaudinn. These all destroy much of the cell-contents, and give an incorrect picture of the cell, excepting chromatinic structures, for which they are indicated.

8. Penetration of Fixatives.

Penetration is the power that a fixing fluid has of passing inwards through the cells forming a tissue. The best penetrators are alcohol, acetic acid and formalin. Less so bichromate of potash and corrosive sublimate. Picric acid also penetrates well. Of the active penetrators, formalin alone is non-injurious to various lipin and fatty substances; acetic acid and alcohol are among the most harmful substances which can be applied to the cell. Osmic acid is a very poor penetrator.

Very few (if any), fixatives penetrate a large piece of tissue quite evenly, and the outside of an organ is almost always fixed differently from the inner regions, because as the reagents soak inwards, some are altered by chemical action, and so the qualities of the fluid become altered in relation to the tissue. In some ways (especially to the cytologist) this may be an advantage, for at certain regions of the tissue the most suitable gravity or strength of the fluid may be arrived at automatically, and so in these regions the cells will be perfectly fixed. It is doubtful how far in fixing mixtures the separate reagents keep together.

The problem of producing some fixing mixture which will penetrate chitin and cyst-walls, and at the same time will not destroy lipins and fats, is still unsolved. Fixatives like Carnoy and Petrunkewitsch are not suitable on account of their alcohol and acetic acid, while formalin does not penetrate sufficiently well for this purpose. (Steam fixation followed by strong chrome-formalin might be a possible method.)

9. General Note on the Use of Osmium Tetroxide (OsO_4).

Osmium tetroxide solution, or osmic acid as it is often called, is the most valuable cytological reagent. Certain substances, such as the unsaturated fat, olein, have the power of reducing the OsO_4, or in other words of being oxidised by the osmic acid. Besides the fats olein, stearin and palmitin, which are almost always mixed in animal tissues, the zoologist is liable to meet lecithin in the form of yolk-discs. Such discs, as is well known, are never pure lecithin, but may contain, besides lecithin, a coagulable protein (vitellin), a free fat and cholesterol. The free fat may or may not always be present in yolk-discs, such as those of frog or snail eggs, but it is a fact that the discs in these eggs generally do not go quite black even when the eggs are soaked for fourteen days in the osmic solution. Mann (**22**) also has noticed that lecithin does not reduce osmic acid; when yolk-discs do go black in osmic acid, it probably means that, not the lecithin, but the associated free unsaturated fat, has reduced the osmium tetroxide solution. The ordinary cytologist, who may be more epecially interested in the morphology and behaviour of certain cell-granules, only recognises fat, lecithin, glycogen or starch as they occur in granular form. It is known of course to most zoologists that the ground protoplasm of the cell contains a number of lipins which do not appear in prepared sections, but which have been detected by extraction or desiccation methods. When, however, such materials are present in such quantities as to form granulations, or to alter the form of the ground protoplasm, they come within the province of the cytologist.

A reaction well known to everyone is that in which the myelin sheath of medullated nerve goes black when treated fresh in osmic acid solution. Cramer, Feiss and Bullock (**8**) affirm that the myelin sheath of medullated nerve contains only such "lipoids" as phosphatides, cerebrosides and cholesterin. The phosphatides comprise such substances as lecithin and kephalin, the cerebrosides are represented by cerebron and the phospho-cerebrosides by protagon. It is obvious that there must be some substance in the nerve-sheath that reduces osmic acid, and this serves to warn us that not every substance which is turned black by osmic acid is unsaturated neutral fat. Kopsch's method for the Golgi apparatus (OsO_4 for two weeks) is another such reaction in all probability, but whether the Golgi apparatus does contain olein we cannot say; what evidence we have points against this assumption.

It is clear that the number of animal substances which have the power of normally reducing osmium tetroxide solution is limited, but it is at the same time equally clear that osmic acid alone is not a specific test for fat. As has been pointed out before, the presence of either chromic acid or bichromate of potash with the osmic acid prevents the blackening of the nerve-sheath or of the Golgi apparatus, but not of animal fat. If a globule in a cell goes black after treatment in Flemming-without-acetic acid, or Altmann, one can safely assume that it is fat or contains fat. In addition the polariscope microscope will be found to help in distinguishing fats and true lipins (see p. 295), and also Cramer (7).

There is no doubt that some of the more capricious results obtained with osmic acid are due to neglect of proper precautions. Capsules and tubes used for the various osmic acid techniques should be scrupulously clean; the water used for making the osmic acid solution should be distilled and as free from dust and other impurities as possible; the solution of osmic acid should be kept in the dark; much discoloured crystals of osmic acid should be discarded, and very old solutions of the salt should not be used. If these precautions are taken it will not be found that OsO_4 gives inconsistent results as certain observers have written.

10. Osmium Tetroxide, Formalin, etc., and Some of their Reactions.

(A) Fat and Mitochondria Preservation.—Animal fat usually consists of a mixture of olein, palmatin and stearin, and osmic acid appears to have the power of converting fat-vacuoles into some substance which is not immediately dissolved out by alcohol, xylol or chloroform. The general reaction of osmium tetroxide is to turn fat black. But this is not all; osmic acid has the power of converting lipins and lecithins into compounds which are insoluble in alcohol, xylol and chloroform. I conclude that this is so by direct experimentation on the granules, fatty or lipoid, found in cells.

The important fact is to be noted that osmic acid is the only fixative which has the power of preserving fat, lecithin, and phosphatide (lipin [mitochondria]) at the same time. Cells containing fat, yolk and mitochondria, fixed in osmic acid and then passed through alcohol (dehydrating) and xylol or chloroform (clearing), are found not to have lost any part of their contents. What, however, do we find with formalin, platinum chloride, bichromate of potash and chromic acid? Fix such a cell in any of these reagents, and pass it afterwards through alcohol and xylol or chloroform, and the fat is removed, while the albumen and phosphatide (lipin, [mitochondria]) remain. Yolk-discs (lecithin) generally remain after this treatment, but are often shrunken. Formalin is particularly active in preserving mitochondria, possibly more so than the chrome salts. It must be noted that these reactions do not mean that formalin and the latter salts themselves destroy fat; it simply means that neither formalin nor the chrome salts are able to act upon fat in such a way as to prevent the latter's being dissolved out in alcohol and the clearing oil; but if one takes a piece of tissue and fixes it in formalin of from 5 per cent. to 10 per cent. or formol-bichromate (as of Regaud), and then treats small pieces of the tissue afterwards in OsO_4 solution, or in chrome-osmium, the fat will be preserved, and will appear in the finished sections. This is an important point for workers on Vertebrate tissues. Thus Müller-formalin, Zenker-without-acetic acid, chromic acid and Zenker-formalin preserve mitochondria and not fat in the finished sections; chrome-osmium, platinum chloride-osmium (Hermann), Kopsch and Altmann, preserve both fat and mitochondria.

(B) Certain fixatives (Ranvier, Kolossow, the old Flemming, etc.) contain alcohol or acetic acid mixed with osmic acid; such mixtures are illogical, because the latter tries to preserve what the former fluids distort or destroy. In certain cases it is found that acetic-osmic acid will dissolve away mitochondria and preserve fat; this is the reverse of what happens in the formol-chrome methods of Regaud or Müller, where mitochondria are preserved and fat extracted. Among invertebrates especially, the acetic acid and osmic fixatives will not preserve both fat and mitochondria, but in some vertebrate tissue the mitochondria are much more resistant.

(C) The reactions of osmic acid with the Golgi apparatus and the mitochondria are rather difficult properly to understand. When animal fat turns black with OsO_4, it has been supposed that the OsO_4 is reduced either to OsO_2, OsO, or to metallic osmium. Another account is that the OsO_4 becomes hydrated to form $Os(OH_2)$. Not claiming to sufficient knowledge of chemistry to enable him to criticise such explanations, the ordinary cytologist can but try to see how such explanations accord with practical cytological technique. What he does find is that a fat-globule not only turns black throughout in OsO_4, but that subsequent dehydration, clearing and embedding (if done carefully) do not cause the globule of fat to shrink in size. Obviously the fat must in some way be altered and made insoluble before use of the dehydratory and clearing agents. The explanation of this is difficult to formulate. One of my chemical friends has suggested that the osmium is brought down to a colloidal condition. I hope that this may be thoroughly examined by a chemist.

If a tissue is left for some time (one to two weeks) in 2 per cent. osmic acid, not only will the fat turn black, but the Golgi apparatus will go quite black, and in the cases examined by me the addition of chromic acid, bichromate of potash and platinum chloride to the OsO_4, while not preventing the fat from going black, does prevent the Golgi apparatus from so doing. In other words, $PtCl_4$, CrO_3 and $K_2Cr_2O_7$ inhibit the special osmic acid reaction for the Golgi apparatus. As an explanation of this, it might be assumed that the blackening of the Golgi apparatus by osmic acid is an oxidising process, but that the admixture of a more powerful oxidiser such as chromic acid to the osmic liquid prevents the latter from carrying out the reaction. Why, however, the Golgi apparatus should not go back under these circumstances, while fat does so, I cannot say. Nor can one say whether the substance of the Golgi apparatus which turns black with OsO_4 is a true fat (olein). Apparently it is a lipin and not a fat.

11. Bichromate of Potash or Chromic Acid with Osmic Acid, and Fats and Lipins.

Osmic acid alone may on rare occasions turn almost any cell element black. This largely depends on whether the osmic solution being used is old or freshly made, and often on certain factors more obscure. In fact the more one uses osmic acid, the more one is led to believe that its reactions are very difficult to understand. This must not be interpreted to mean that it is impossible to get reliable results by using osmic acid. It was mentioned before (**12**) that Flemming fluid without acetic acid does not blacken the Golgi apparatus in mollusc gonads, while osmic acid alone will. In the Marchi method to demonstrate degenerate nerves, parts of the nervous system containing regions of degeneration are fixed in Müller ($K_2Cr_2O_7$) ; they are then treated in $K_2Cr_2O_7$ and OsO_4, and it is found that the normal medullated sheaths will not go black in the OsO_4, while the diseased ones will. As is well known, osmic acid alone will turn the normal sheaths black. The treatment with the chrome-salt prevents this reaction taking place in all but the degenerate sheaths. In nerve degeneration it appears that glycerides of unsaturated fatty acids are formed apparently at the expense of the lipins which constitute the normal medullated nerve-sheath. It is this unsaturated non-phosphorised fat which, according to

Cramer, is responsible for the "Marchi reaction." For the zoologist the importance of this is very considerable; any substance in a tissue which goes black in Altmann, Hermann or Flemming-without-acetic is almost (though not quite) certain to be fat, or a glyceride of the unsaturated fatty acid such as olein.

The zoologist working on development and cytology in general is satisfied if he is able to identify any substance as "fat" or "oil," and distinguish it, if possible, from yolk-spheres or discs. Cramer considers that the sheath of the medullated nerve contains no unsaturated fat or fatty acid, but only lipoids such as lecithin, protagon and cholesterin, and he maintains that all turn black with osmic acid. Not being a skilled histo-chemist, I feel that it may be injudicious for me to say that I doubt Cramer's statement. Mann (**22**) states that lecithin (term used widely) does not become black with OsO_4, and I find that the yolk-discs of frog's and mollusc's eggs do not go black in osmic acid. I suggest that the substance in the sheath of medullated nerve which is blackened by OsO_4 is not lecithin; it may be cholesterin or protagon—I feel unable to comment on whether these substances do turn black in OsO_4; the only occasions yolk-discs immediately go black in osmic acid is when they contain associated unsaturated fats (Aves) or after Sjövall's method (**13**). In explanation of the "Marchi reaction" and the chrome-osmium blackening effect on fat and not on the Golgi apparatus it may be assumed that of the two oxidising reagents, the chrome salt is the most effective in the case of the Golgi apparatus and with lipoids generally, but does not oxidise the fat completely if at all.

12. Osmic Acid Vapour Fixation.

It is well known (**3**) that osmic vapour is very penetrating and toxic, and rapidly fixes smears and small pieces of tissue. My friend Dr. Cramer tells me that adrenalin granules in the suprarenal glands are best demonstrated by suspending a small organ (or a part of it) in a slightly wet iron gauze bag, over osmic acid in a glass-stoppered tube at 37° C. for one and a-half hours. By this method the vapour penetrates into the tissue without the addition of any water. The main point to note is that substances in a tissue which might be dissolved out or altered by the water added to the OsO_4 crystals are fixed in sitû, and without the danger of alteration. This method should be of value to histologists and cytologists. Cramer directly transfers his fixed tissue to 50 per cent. alcohol and then up grades to absolute alcohol and mounts sections unstained. My three modifications of this method are as follows:

(A) Fix as above for one and a-half hours and then transfer to 2 per cent. OsO_4 in water at 37° C. for one to several days. Then wash in water for several hours, dehydrate, embed and section. Mount unstained, or stain in acid fuchsin (Altmann) or iron-hæmatoxylin.

(B) Or fix as above for one and a-half hours and transfer to 1 per cent. chromic acid, or 3 per cent. $K_2Cr_2O_7$ for several days. Wash several hours in running water and stain in acid fuchsin, or mount unstained.

(C) Tissues may also be fixed as above, and then transferred to Altmann's fluid, and subsequently stained in Altmann's fuchsin and picric acid. These methods should be useful to protozoologists. (See section on post-chroming.)

13. New Modifications of Kopsch's Method for the Golgi Apparatus, etc.

In my previous paper (**13**) I gave Kopsch's method. It consists of leaving small pieces of fresh tissue in 2 per cent. OsO_4 for from one to two weeks at room-temperature, then washing, dehydrating, and embedding. Sections mounted without staining show the Golgi apparatus and the fat (sometimes yolk) black. Mitochondria rarely go black, generally yellowish. This unmodified method is quite good for Mollusca, Amphibia, and Mammals. It gives poor or often completely negative results for Arthropoda. Moreover, Kopsch's unmodified method generally causes bad shrinkage. The new methods are as follows:

A. Primary fixation in formalin (Sjövall) of a suitable strength (circa 5–10 per cent.), followed by slight washing in aq. dest.	Secondary treatment for several hours or one or two days in OsO_4 of 2 per cent. Then wash in distilled water several hours and dehydrate.
B. Primary fixation in Mann's corrosive osmic (**22**) or corrosive alone for two or three hours, followed by aq. dest. as before.	Secondary treatment for from one to several days up to two weeks in OsO_4 of 2 per cent. Wash as above.

To increase the power of method B, the secondary treatment may be carried out in thermostat at a temperature from 20° C. to 40° C. for one or two days.

Note also Kopsch-Altmann, mentioned in my previous paper (**13**).

14. The Flemming-without-Acetic Acid and Iron-Hæmatoxylin Technique.

Flemming-without-acetic (F.W.A), Altmann or Champy, are chrome-osmium fixatives, which are admirable when followed by iron-hæmatoxylin or Altmann's acid fuchsin and picric acid. Since my most successful preparations have been procured with the F.W.A. iron-hæmatoxylin technique, it may be useful to other workers to give details of the process.

Small organs freshly dissected out in normal saline, or parts of organs cut with a sharp scalpel, not more than 5 mm. in diameter, are placed in about 15 c.cm. of one of the above-mentioned fixing fluids. A glass-covered capsule is the best vessel to use, and the material is left for at least twenty hours, and not longer than one week. I find about twenty-four hours gives a satisfactory fixation of most tissues. After fixation the liquid is poured away, and the material is washed for at least two hours, and not necessarily longer than five, in running tap-water. It is then passed through up-graded alcohols, beginning at 30 per cent., giving the material at least three hours in the strengths 30 per cent., 50 per cent., and 70 per cent., and overnight in 90 per cent. The pieces of tissue are dehydrated two or three hours in two changes of absolute alcohol, and then transferred to a mixture of half absolute alcohol and half xylol for one quarter of an hour. They are left in pure xylol for another quarter of an hour, and then a mixture of cold wax and xylol is thrown into the capsule. The latter is transferred to the thermostat and left for half an hour, and then the liquid is poured away and pure melted wax is added. This is left for an hour, and the block is prepared and cooled in the ordinary way. Sections are cut from 4 to 8 μ, but I generally find 6 μ to be convenient. Sections are stuck on the slide with dilute glycerine and albumen water, and left overnight on the warm plate. The wax is melted off in xylol, and the sections are brought through alcohols to distilled water. They are mordanted from six to ten hours in 4 per cent. iron-alum, washed once or twice in distilled water, and transferred to hæmatoxylin of $\frac{1}{2}$ per cent. for ten to fifteen hours. They are then washed in tap-water, differentiated in the iron-alum to the correct degree and washed again for five minutes, and then dehydrated and mounted; or they may be counterstained in orange G. For Altmann's acid fuchsin and picric acid stain, a previous fixation or a post chroming in some fluid containing bichromate of potassium gives the best results; Altmann's fluid is admirable. (See section on post-chroming.)

15. Formalin and Iron-Hæmatoxylin.

For a microchemical test, and for clearly demonstrating the mitochondria, formalin and iron-hæmatoxylin are very good.

To 25 parts of formalin add 75 parts of distilled or tap-water (the latter is often more desirable). Neutralise the solution with magnesium carbonate. Leave pieces of tissue about the size of one's small finger-nail, in 20–30 c.cm. of the fluid for two days. Wash for half an hour each in 30 per cent. and 50 per cent. alcohol and leave for two hours in 70 per cent.; transfer to 90 per cent. and leave overnight. Finish dehydration, clearing and embedding, and stain sections in iron-alum hæmatoxylin—alum three hours, hæmatoxylin from five to nine. In this connection see also Regaud's (**28**) fixation method (p. 289) which is admirable for mammalian tissues and gives splendid results when followed by iron-alum hæmatoxylin. After Regaud fixation, dehydration need not be carried out so rapidly.

16. Post-Chroming (Mordantage).

To get a good Altmann stain and often to get any sort of satisfactory stain of mitochondria one must post-chrome, if the tissue has not been fixed in a mixture containing bichromate of potassium.

Material may be first of all fixed in formalin, corrosive sublimate, Zenker, chrome-osmium or such fixatives as those not containing alcohol, acetic acid and chloroform, and subsequently left for three to seven days in 3 per cent. potassium bichromate, then washed under tap overnight.

In addition smears or tissues may be fixed in osmic or formalin vapour for some time (one to two hours), and then left several days in the bichromate, washed overnight in running water, and then dehydrated, giving at least five hours to each strength; in some cases ten hours gives better results. The tissue may then be embedded, or smears may be brought down again to water and stained. Post-chroming is used in the methods of Benda and in the latest modifications of Altmann. The point to be noted here is that the well-known mitochondrial stains can thus be used even if tissue has not been first fixed in a special mitochondrial mixture.

Another post-chroming method, which mordants tissues prior to staining, is to soak the material in a mixture of pyroligneous acid (1 part) and chromic acid of 1 per cent. (2 parts) for one to several days. Then transfer the material or slides to 3 per cent. $K_2Cr_2O_7$ for from three days to a week.

17. Osmicated Fat, etc., and Turpentine.

Osmic acid fixatives have the disadvantage, in working with materials containing much fat or yolk, of blackening and obscuring the sections. Other cell elements may be obliterated by a cloud of black or brown granules of fat. Since many of the modern techniques for mitochondria and Golgi apparatus consist partly in the use of osmic fixatives, this is a serious difficulty. It can be got over quite easily, however, as my friend Dr. J. A. Murray has pointed out to me, by soaking the sections on slide in turpentine oil, which has been left in sunlight (Bolles Lee, **3**). Sections are prepared by any special osmic or osmic-chrome method, the wax is melted off them in xylol, and they are then transferred to a jar of turpentine. In some cases the black osmicated fat-globules come away in streams almost immediately ; in other cases the process must be carried on overnight. When this method is tried on yolk-grains such as those of mollusc gonads, the brown or black colour is removed after a few hours and the yellowish disc or sphere remains. After treatment in turpentine the mitochondria can be stained in iron-alum hæmatoxylin.

I find that the Golgi apparatus in the gut-cells of the rat and of the newt (Molge) resists this treatment and remains black, while the fat- and yolk-granules are extracted and decolorised respectively. This method is important, and should be carefully noted by those who have found the diffuse blackening of cytoplasmic granules in osmic fixation to be a disadvantage. It can also be used to help in discrimination between Golgi granules and fat or yolk.

18. The Acid Fuchsin Methyl-Green Modifications of Altmann's Mitochondrial Stain.

Altmann's acid fuchsin and picric acid stain has in my hands given satisfactory results, and I have not noticed that it faded much. By it mitochondria are stained reddish to reddish-purple according to the sample of fuchsin used, while the cytoplasmic background is yellowish with a reddish tinge. The nucleus is reddish. Bensley (**2**) substitutes methyl green for the picric acid, and according to Cowdry (**6**) this modification of Altmann's method is one of the most specific stains for mitochondria. The latest modification used by Cowdry (6) is rather complicated and long; but provided a good specimen of acid fuchsin is obtainable the result is excellent and the stain can easily be learnt.

I regard Altmann's, Kull's or the Bensley-Cowdry stain as a natural and useful alternative to iron-hæmatoxylin. Regaud's mixture gives excellent results followed by iron-hæmatoxylin, so both methods can be used for sections cut from the same block. The same applies to Harry Kull's method given below (p. 290).

(A) Cowdry's Modification of the Altmann-Bensley Stain (Bensley-Cowdry).—(1) Fixation.—Regaud's mixture, 4 parts of 3 per cent. $K_2Cr_2O_7$ and 1 part commercial formalin (neutralised by saturation with magnesium carbonate). Change mixture every day for four days; mordant for eight days in 3 per cent. $K_2Cr_2O_7$, changing every second day.

(2) Wash in running water overnight.

(3) Dehydrate, clear and embed in wax; cut 4 μ.

Staining.—(1) Pass slides down through toluol, absolute alcohol, 95 per cent., etc., to water.

(2) Dip in 1 per cent. potass. permanganate thirty seconds or so.

(3) Rinse in 5 per cent. oxalic acid thirty seconds. (Note: Steps (2) and (3) may usually be dispensed with.)

(4) Rinse in distilled water about one minute or longer.

(5) Stain in Altmann's anilin fuchsin as follows: Make a saturated solution of anilin oil in distilled water by shaking the two together. Filter, and add 10 grm. of acid fuchsin to 100 c.c. of the filtrate. The stain is ready in twenty-four hours, but goes bad in a month. Dry around sections with duster, cover sections in fuchsin, and heat over lamp till anilin fumes steam off; allow to cool; let stain remain on about six minutes, and return former to bottle.

(6) Blot; rinse in distilled water as long as is experimentally found convenient.

(7) Pipette a little 1 per cent. methyl green over sections

for some five seconds or so as indicated. This is the crucial point of the method.

(8) Drain; transfer to 95 per cent. alcohol for a second or two. Absolute, toluol, balsam.

The next method, that of Harry Kull, will be found easier and quicker in some ways ('Anat. Anz.,' xlv, 1913).

(B) New Modification of Altmann's Method (Kull's Method, or Champy-Kull).—The following method, while being generally useful, will be found very convenient for work on Invertebrata. It gives results intermediate between that of Benda and Altmann, but is shorter and possibly better than the method of Benda. It will be found very useful for embryological research, and probably also for protozoology: Fix in Champy for twenty-four hours. Champy's fluid is as follows: Seven parts 1 per cent. chromic acid, 7 parts 3 per cent. bichromate of potash, and 4 parts of 2 per cent. OsO_4. Pieces to be fixed must be small. After fixation wash half-an-hour in aq. dest., and then transfer to a mixture of 1 part acid. acet. pyrolignosum rect., and 2 parts 1 per cent. chromic acid for twenty-hours. Wash half an hour in aq. dest. and transfer to a 3 per cent. solution of potassium bichromate for three days. Wash under tap for twenty-four hours; pass through up-graded alcohols to xylol; embed in paraffin wax (or celloidin method if desired). Section 4 or 5 μ.

Stain as follows: (1) Stain in Altmann's acid fuchsin aniline oil mixture (20 grm. of acid fuchsin in 100 c.c. of aniline oil water) and heat till steaming.

(2) Set slide aside to cool (this is important), pour off, and wash in aq. dest.

(3) Counterstain in either a 0·5 per cent. solution of Toluidin blue or a saturated solution of thionin in aq. dest. for one to two minutes.[1] Wash in aq. dest.

(4) Differentiate in a 0·5 per cent. solution of aurantia in

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[1] I find less time than this sufficient. Moreover, in my hands the differentiation period is not during paragraph (4), but during subsequent treatment in the 96 per cent. alcohol. This is the best stain, other than iron hæmatoxylin, that I have used.

70 per cent. alcohol for from twenty to forty seconds, watching process under microscope.

(5) Wash in 96 per cent. alcohol, then absolute, xylol, and balsam.

The chromatin is blue; mitochondria are red, and the ground cytoplasm is golden-yellowish to green.

19. Remarks on Silver Nitrate Methods.

One of the oldest methods for the demonstration of cell-walls is the silver nitrate technique. Fresh tissue is treated with $AgNO_3$ solution, and the salt is reduced in sunlight. This method is fairly capricious, and the preparations so produced are liable to after-blackening. The Golgi apparatus of Vertebrata especially can be demonstrated by the methods of Cajal and Golgi; these methods consist essentially in the fixation of a tissue in formalin followed by a bath of silver nitrate solution; the nitrate is reduced and then toned. Those who have not used these methods and whose knowledge of silver nitrate methods consists of an acquaintance with the old $AgNO_3$ technique for cell-walls are liable to be led away wrongly by the view that Golgi and Cajal's methods are capricious and untrustworthy. I find, and several of my co-workers have found more recently, that these methods are remarkably uniform in their results provided proper care is taken. No histological method gives completely uniform results, but to identify the bodies which one sees in Cajal and Golgi preparations as artefacts is absurd. That this is so I hold for several reasons: Firstly, the methods are very uniform in result, and give the same results on many different sorts of animals; secondly, the methods of Cajal and Golgi demonstrate the bodies which are shown by OsO_4 (Kopsch's method); thirdly, these silver methods impregnate bodies, which in the case of germ-cells may be seen in the living cell, or stained with intra-vitam stains. Lastly, these methods demonstrate bodies which can be, and have been, followed through the germ-cell cycle in all stages. These facts cannot be refuted by critics of the silver nitrate methods, and are, I believe, conclusive.

20. The Golgi Apparatus Method of Golgi.

In my former paper (**13**) on technical methods I gave Cajal's Golgi apparatus technique. Another method, closely similar in some ways, is that of Golgi; both of these techniques consist in the fixation of the tissue in some fluid containing formalin, then immersion of the fixed material in $AgNO_3$ for some time, and subsequent reduction of the silver salt in a fluid containing hydroquinone; in a later stage excessively reduced silver is toned in "hypo" mixture. Two main difficulties in such techniques are firstly to attain just the right length of time for soaking the material in the silver nitrate, and then secondly to reduce the silver just to the correct stage. After one or two trials subsequent attempts generally succeed.

A. Golgi first fixes six to twenty-four hours in the following mixture:

Formalin of 20 per cent.	30 c.c.
Saturated sol. arsenious acid	30 c.c.
Alcohol of 97 per cent.	30 c.c.

B. Tissue transferred to 1 per cent. $AgNO_3$ for from one hour to several days.

C. Reduced for two or three hours in mixture:

Hydroquinone	20 grm.
Sodium sulphite	1 grm.
Formalin	20 c.c.
Aq. dest.	1000 c.c.

D. After this the pieces of tissue are washed about ten minutes in distilled water and then dehydrated as quickly as possible, and embedded in wax or celloidin and cut about 6 μ.

E. Sections on slide are toned (differentiated) in following mixture:

(1)	Sodium hyposulphite	30 grm.
	Ammonium sulphocyanate	30 grm.
	Aq. dest.	1000 c.c.
(2)	Gold chloride	1 per cent.

Mix equal parts of solution (1) and (2) just before use. Tone the sections till the cells look clear and the Golgi elements stand out. Wash in aq. dest., dehydrate, clear, mount in balsam (see Pappenheimer, **25**).

As will be noted the fixative contains a good deal of alcohol which I consider bad technique. While Golgi's method may give fine result for mammalian material, there is no doubt that Cajal's fixative (**4**, **13**) produces a better result for more delicate tissues.

Some of the material after fixation in Cajal or Golgi formalin mixture can be treated as for the formalin and iron-hæmatoxylin method on p. 286. I find such fixation to give very satisfactory results apart from the impregnation of the Golgi apparatus.

21. Chromosome Work and Urea.

It has lately been claimed by Hance (**16**) that the addition of about ½ per cent. urea to fixatives of the Bouin or Flemming type prevent the clumping together of chromatin structures such as chromosomes or chromatin filaments, and enable the worker to count with ease such difficult chromosome plates as those of Vertebrata. My personal experience of such modified fixatives is at present not extensive enough to permit me to express an opinion. (For a reference to this see 'Journn. Roy. Micr. Soc.,' 1917, p. 347.)

22. Vertebrate and Invertebrate Tissues.

It is very rare to find that one single method will produce the same good result in both Vertebrate and Invertebrate tissues. In the same way, methods which act satisfactorily with Amphibia will often give disappointing results with Mammalia. Osmic-chrome fixation will nearly always be found excellent for all classes of Invertebrata; Flemming-without-acetic acid can be highly recommended. For Amphibia the addition of some $K_2Cr_2O_7$ to the Flemming is necessary before a correct fixation of the mitochondria is obtained; thus Champy's fluid was invented for Amphibia and gives very satisfactory results.

For mammalian tissues a preliminary fixation in osmic acid fixatives is not generally indicated; the tissues of mammals are far more "fatty" than those of Invertebrata or Amphibia, and one finds that the OsO_4 becomes reduced very rapidly and penetration is very poor. For mammalian tissues formalin-chrome or formalin alone are indicated as a preliminary treatment at the least. Formalin does not destroy lipins, and by subsequently placing small pieces of formalin-fixed tissues in osmic acid, a fixation of lipins and fats is obtained; the same result may be got by fixing tissues in chrome salts and then transferring to osmic acid. It should be noted, however, that previous fixation in a chrome salt prevents the blackening of the Golgi apparatus; the formalin-osmic acid method (Sjövall) is indicated especially for mammalian tissues, when an impregnation of the Golgi apparatus is required by means of an osmic method, and the formalin silver nitrate techniques always give results of value.

23. Dark-ground Illumination and the Cell Inclusions.

While it is true that very small micro-organisms can be conveniently seen with the well-known dark-ground illumination, the problem of studying the mitochondria and Golgi elements by this means is a difficult one. A spirochæte or bacillus floating in blood-plasma lies in a fluid substance of a different refractive index, while a mitochondrium or a Golgi rod is generally of nearly the same refractive index as the protoplasm in which it lies; in Limnæa eggs the mitochondria are easily seen with the ordinary microscope illumination because they are impregnated with a lipochrome, but under dark-ground illumination such granules are less easily seen. The Golgi (Nebenkern) elements in Helix spermatocytes are tolerably well seen in the fresh cell under ordinary direct illumination, because they differ slightly in refractive index from the protoplasm, but in the nerve-cell the Golgi elements are to my knowledge imperceptible under the same conditions; yet both Kopsch and Golgi or Cajal techniques demonstrate a Golgi apparatus in both Helix spermatocyte and nerve-cell. In the former case direct observation reveals that such staining techniques impregnate bodies which can be seen intra vitam.

With the most critical conditions the mitochondria and Golgi elements can be studied in the live cell using dark-ground illumination, but this method will not replace fixation and staining techniques, and at best merely shows granules as pale rings of light.

24. The Polariscope Microscope as an Adjunct to Cytological Research.

Some help in distinguishing between or merely detecting substances in the cells of tissues may be gained by the use of a polariscope microscope. The theory of this instrument can be read elsewhere (**31**), but it is well known that those substances, which are singly refracting, are quite dark between crossed nicols and remain so during a complete rotation of the stage, and are called isotropic, while those substances which show themselves as points of light between crossed nicols and are alternately dark and illuminated when the stage is rotated are known as anisotropic. True neutral animal fat is isotropic, while certain lipins are anisotropic (**7**). The development and use of this method of examining zoological material has as yet been inextensive, and it is possible that such a method of examining fresh eggs and embryos may yield some interesting facts when used in conjunction with other cytological methods.

Polarised light will not produce a result in every case; one of my most perfect Kopsch (OsO_4) slides of the Limnæa ovotestis, which under the ordinary microscope revealed mitochondria, Golgi apparatus and yolk-discs very clearly, was quite isotropic. This may be due to the OsO_4 altering the optical properties of the intracellular materials. Ovaries and testes may be teased out fresh in their own juice and examined intra vitam. This gives the best result, and possibly the only reliable one.

25. General Plan for Cytological Research.

The zoologist meets with the following bodies in the cell Nucleus, and in the cytoplasm, Golgi apparatus, mitochondria, fat, yolk, and glycogen. He may also find pigment-granules.

(1) Some of the material to be worked on must be fixed in chrome-osmium, of which fluids Flemming-without-acetic acid, Altmann, and Champy may be tried. In testes, ovaries and in eggs this is certain to preserve nearly all the cell elements, i.e. mitochondria, Golgi apparatus, fat and yolk. It is doubtful whether glycogen is preserved. On staining, the mitochondria will go black in iron-hæmatoxylin, or red in acid fuchsin (Altmann, Champy-Kull, or Bensley). The Golgi apparatus may stain in the case of the male germ-cells, but not generally in the egg or embryo; yolk is generally yellowish green-brown, or more rarely black, and remains so even after the Altmann stain. On staining in iron-hæmatoxylin yolk may or may not turn black; in the frog this stain turns yolk black, in the snail it does not do so. In these preparations fat goes black also; but fat may easily be distinguished from other elements by mounting some sections unstained, or simply by staining in acid fuchsin (Altmann). In these preparations what is black is almost certain to be fat or contain fat, because chrome-osmium alone, so far as my experience goes, never turns the Golgi elements or mitochondria black. By this chrome-osmium fixation one gets a good preliminary survey of the mitochondria and other cell elements.

(2) The next stage may be taken as follows: Some tissue is fixed about two days in 5–10 per cent. formalin, which should be carefully neutralised in magnesium carbonate. Dehydration and clearing is carried out in alcohol and xylol respectively. Staining should be done in iron-hæmatoxylin. The result of this technique is to get rid of fat, but to stain mitochondria densely black. In eggs and embryos the Golgi apparatus generally does not show, but it may do so in the male germ-cells. The effect of fixing material in chromic

acid or bichromate of potassium with formalin is nearly the same as with formalin alone; none of these reagents has the power of making fat insoluble in alcohol + xylol, but all have the power of making mitochondria insoluble in the same reagents. In formalin yolk is preserved, and may go black in iron-hæmatoxylin, but I do not know of its going red in acid fuchsin. Fat is lost.

(3) The next step is to attack the problem of the Golgi apparatus. Two methods should be tried.

(A) Kopsch's osmium tetroxide methods, in which small pieces of tissue are treated in the osmic fluid (2 per cent.) for from one to fourteen days, then sectioned and mounted unstained, or stained in acid fuchsin (Altmann). The Golgi apparatus will go black; by this method fat also will go black, and yolk may do so. The mitochondria almost always remain yellowish or unstained by the osmic fluid, and subsequent treatment of the material in acid fuchsin will turn these granules red. The Golgi apparatus remains black (see also various Kopsch modifications, p. 284). Such preparations can with advantage be compared and contrasted with those made by chrome-osmium methods as in paragraph (1).

(B) The other method which should be used is that of Golgi or Cajal. In my previous paper on this subject (**13**) I gave the method of Cajal, and I have described that of Golgi here (p. 292). Cajal's method differs from Golgi's in one very important respect, i. e. that the latter's fixing fluid contains a good deal of alcohol. Both Golgi and Cajal's silver methods for the "apparato interno reticolare" are progressive, and in one block of tissue one may find regions where no "apparato" is impregnated, regions where only the Golgi apparatus is impregnated, and regions where both Golgi elements and mitochondria are black. In typical cases, however, the latter go yellowish to brown. Material fixed in Cajal or Golgi fixative may be stained in iron-hæmatoxylin.

(4) As a general rule it is advisable to fix and stain for glycogen. There are two methods—the iodine (**13**) and Best's carmine. Details of these will be found either in Bolles

Lee's 'Microtomist's Vade-mecum' or Mallory and Wright's 'Pathological Technique.'

(5) Some material should be fixed in a fluid such as that of Bouin, Carnoy, Gilson-Petrunkewitsch, Zenker or Flemming-with-acetic acid, and stained in Ehrlich's hæmatoxylin and eosin, or in iron-hæmatoxylin and orange G. These preparations are useful for details of spindle-fibres, centrosome, and especially for ascertaining oxyphility and basophility of ground cytoplasm, or of nuclear bodies. Such preparations are also a sine quâ non where a study of chromosomes is to be carried out, because chrome-osmium and other mitochondrial fixatives not containing alcohol and acetic acid do not generally give a sharp picture of chromatinic structures. For ascertaining oxyphility, amphophility or basophility staining in Ehrlich's hæmatoxylin and eosin or Biebrich scarlet gives good result. This method was perfected by the late Dr. S. G. Scott of the Histology Laboratory, Oxford. Its main advantage is that it gives a correct histological picture of the degree of oxyphility or basophility of any cell body or of the ground cytoplasm, without overstaining and washing out, which introduces complications.

(A) Bring sections to 90 per cent. alcohol and wipe slide dry except around sections. Add just enough drops of Ehrlich's hæmatoxylin to cover sections. Leave slide on bench for ten minutes.

(B) Drain away hæmatoxylin, and wash off every trace of latter with 90 per cent. alcohol, and then with aq. dest.

(C) Blue the sections in the following standard tap-water substitute:

$KHCO_3$	2 grm.
$MgSO_4 + 7H_2O$	20 grm.
Aq. dest.	1000 c.c.

Add lump of thymol.

Leave some drops of this mixture on slide for three to four minutes, and then wash in distilled water thoroughly.

(D) Wipe around sections, and add some drops of weak eosin (1 in 1000 in aq. dest.); leave ten minutes till pink or reddish (control under microscope). (Scott uses particularly Biebrich scarlet instead of eosin, as the former is less diffuse.) Drain off stain, quickly wash in aq. dest.

(E) Blot, dip into absolute alcohol and quickly transfer to xylol. Mount in balsam.

(6) During the preparation of these sections it is advisable to examine both fresh and intra-vitam stained teased preparations. The animal is killed by a blow on the head, or if an invertebrate, by cutting off its head, the gonads or other organs are rapidly removed and pieces are teased in the animal's own juice (lymph), or less preferably in normal saline, and examined as soon as possible. After examination of fresh cells (or eggs) the teased material may be examined as follows:

(A) Freshly teased cells to which Janus green has been added (Janus green in normal saline solution, 1 in 20,000 to 1 in 30,000). (Results depend greatly on the particular specimen of dye.)

(B) Ditto in neutral red, 1 in 10,000 to 1 in 30,000.

(C) Ditto in OsO_4 of 1 per cent., and in weak permanganate of potassium. (See also **13**.)

(7) In addition the dark-ground illumination and the polariscope should be tried, but it should be noted that neither of these contrivances at present provide the researcher with extensive lines of evidence.

26. Conclusions.

In these notes I have indicated the general trend of the more modern cytology. In the previous paper I gave tables and figures which should enable workers not familiar with the cytoplasmic inclusions to identify any given body. The last few years have seen a great advance in technique, and workers should now be able to classify and follow separately any given substance or material in the egg or embryo, as well as in the gland.

It should not, however, be considered that the problem of the cell organs and granules is solved—far from it—for there are (especially in gland-cells and protozoa (**10**)) enigmatic bodies, possibly of an obscure metabolic nature, whose true identity has not been established. Nevertheless, I consider that such rapid advances have been made that there are now few cell bodies which the zoologist cannot easily identify and follow during organogeny and gametogenesis.

What is now wanted, and would be of incalculable value, would be some method which would reveal chromatin in all its stages, and which would enable us to follow out the interchanges between nucleus and cytoplasm.

UNIVERSITY COLLEGE, LONDON;
June 20th, 1919.

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On the Spermatogenesis of the Louse (Pediculus corporis and P. capitis), with some Observations on the Maturation of the Egg.

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With Plate 16 and 1 Text-figure.

1. Introduction.

The work described in this paper was undertaken in the hope of throwing some light on certain curious phenomena with regard to sex which had occurred in breeding experiments carried on with the body- and head-louse by Hindle and by Bacot. Hindle (**6**, **6a**) observed that among the offspring of single pairs of lice, although frequently both sexes appeared in about equal numbers, quite often the whole family reared consisted either only of males or only of females, or in other cases was almost entirely of one sex, with one or two offspring of the other. He also found that when a female, which, with one mate, had given a family of a certain type, was paired with a different mate, the family might be of another type, e. g. a female mated with male A gave only male offspring (fourteen ♂ ♂), but when mated with male B she gave both males and females (three ♀ ♀, one ♂). There were also indications that the same male paired with two different females might have offspring of different sexes.

The second remarkable observation in regard to sex was made by Keilin (**7**) on material reared by Bacot. Bacot crossed the male head-louse (P. capitis) with the female body-louse (P. corporis), and on making an anatomical examination of some of these hybrids Keilin found that a number of them were analogous to the "intersexes" obtained by Goldschmidt (**4**, **5**) in the hybrids between different races of the moth Lymantria (Liparis) dispar. These "intersexes" can hardly be called gynandromorphs—a name which implies a sharp division of the body into regions having male and female characters, but are rather an intimate blend or fine mosaic of male and female organs or characters. Since Goldschmidt has based very fundamental conclusions with regard to the physiology of sex-determination upon his study of the intersexes of L. dispar, and extremely few other instances of the kind are hitherto known for comparison, it seemed that a thorough study of the cytology of the two parent species or varieties, and if possible of the "intersexual" hybrids, was very desirable.

During our investigation a number of other points of interest appeared, some of which are dealt with here and to some we hope to return in a later paper. External events have at present interfered with the continuation of the work, and we have not been able to obtain intersexes for examination, so that the present paper makes no pretence of elucidating the questions in regard to sex which led to our beginning the work. The cytology of the spermatogenesis, however, is so remarkable, and in some respects so unlike anything known in other forms, that we think it advisable to publish our results on the pure species for their own sake and to return to the subject in a later paper if we are able to do so.

2. Methods.

The method adopted for breeding the lice required was that used by Nuttall. The lice (P. corporis) were kept in small, round cardboard boxes, in the bottom of which a hole about ¾ in. in diameter was punched and covered with fine muslin. Each box contains a piece of cloth for the lice to rest on. The boxes were kept in an incubator at about 30° C., and were fastened with an elastic band on the wrist or leg for half an hour twice each day. The lice feed readily through the muslin. A number of boxes can be worn at once without interfering with the activities of the wearer, and for most people, unless there are very many lice in a box, hardly any discomfort is caused. P. capitis feeds less readily, and the boxes containing it should be worn on the person continuously.

For preserving testes or ovaries for cytological examination, it was found that the simplest method was to place the louse in a drop of water on a glass slide under a mounted lens, behead it with a needle, and while holding the thorax by means of a needle with one hand, cut off the tip of the abdomen with a scalpel or sharp needle with the other. By light pressure on the abdomen from before backwards with a needle it was then quite easy to squeeze out the whole contents, and to place the whole mass, from which the testes or ovaries usually protrude conspicuously, in fixative. In male nymphs of Stage I, even immediately after hatching, the testes are almost always clearly visible by this method as paired bodies like two pears placed with their thicker ends together; in female nymphs of Stages I and II the ovaries are less easily seen, but can usually be found without much difficulty with a little teasing. It is thus possible to record the sex of nymphs, especially of Stages II and III, quickly and accurately by this method, without spending the time and trouble needed for rearing them to a stage when the external sexual characters become visible.

Several fixatives were tried, but with testes much the best results were obtained by Flemming's strong solution or modifications of it. Experiments were also made of dissecting the lice in water, salt, or in Ringer's solution, and in Flemming, but in general water gave the best results. For the study of chromosomes most of our material was fixed in Flemming without modification, but since in some stages the chromosomes are closely packed together and difficult to see separately, we tried Flemming with twice and three times the normal proportion of acetic acid, in the hope of spreading them by causing the cytoplasm to swell; in some cases we got clearer figures of chromosomes and of axial filaments of developing spermatozoa by this method. For the study of mitochondria (which, however, are often well shown in testes of Pediculus fixed with normal Flemming) we used chiefly Flemming without any acetic ("F.w.a."), either of normal strength or diluted with water. Flemming without acetic, diluted with three times its volume of water ("F.w.a. $\frac{1}{4}$") gave particularly good results. We also tried the method recommended by Champy of treating material fixed in "F.w.a." (full strength) followed by 3 per cent. potassium bichromate for forty-eight hours at 50° C., without, however, finding any improvement. The ovaries of older females were well fixed with such sublimate mixtures as that of Gilson. Most of our sections were stained on the slide by Heidenhain's iron-hæmatoxylin method. Several other stains and combinations of stains were used for comparison, usually without any great success in elucidating points which the hæmatoxylin preparations left obscure. Some of these will be referred to below.

The description which follows is taken from our preparations of P. corporis, but we have found no essential differences between P. corporis and P. capitis.

3. SPERMATOGENESIS.

A. General Outline.

The testes are already well developed, though small, in nymphs immediately after they hatch from the egg. At this stage, and throughout Stage I—i.e. before the first moult—they contain only spermatogonia.[1] These cells are arranged in groups or follicles, an arrangement which is not very evident among the resting cells, but is clearly seen when all the cells in one follicle are in division while the surrounding cells all have resting nuclei. They probably divide a number of times, for in young Stage II nymphs, i.e. after the first moult, the testes contain many more cells, some of which are now beginning to increase in size and are becoming spermatocytes. This takes place chiefly at the thicker end of the testis, nearest the vas deferens, and even in Stage III, spermatogonia like those of Stage I remain at the distal end and for a considerable distance along the sides of the testis, while the interior is filled with spermatocytes and later stages.

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[1] The question whether the cells at this stage are spermatogonia or should properly be called primary spermatocytes will be discussed below.

During the growth-phase the spermatocyte enlarges considerably and the mitochondria become conspicuous; there is at this stage a large nucleolus. At the close of the growth-phase the mitochondria coalesce to form a conspicuous "mitosome," which usually appears vacuolated. At the same time a deeply-staining round body appears near the nucleus, which we call the acroblast, since we believe it is concerned with the formation of the acrosome of the spermatozoon; it will be discussed more fully below.

After the growth-phase the spermatocytes undergo a division which is remarkable in two respects. In the first place, it is the only spermatocyte division, for as far as we have been able to discover no second division occurs; and in the second, it is strikingly unequal as regards the cytoplasm of the cell, resulting in the separation of a nucleus with a small amount of cytoplasm from a relatively large cell which retains the whole mitochondrial body and the "acroblast." The division is in most respects much like the single spermatocyte division of the honey bee, and, as in the bee, the small cell degenerates without developing further, while the large cell is converted into a spermatozoon. The most remarkable feature of the later stages is that the centrosome of the spermatid, as the large cell must now be called, is paired, and from each centrosome an axial filament grows out, with the result that in its latest stages each spermatid has two very conspicuous axial filaments. The behaviour of the mitosome and "acroblast" will be described below.

B. The Nucleus and Chromosomes.

In cells of the body of both males and females, and in certain cells of the testes which are conspicuously larger than the spermatogonia, and which, from their occurring singly between the groups of spermatogonia, are doubtless follicle cells, the chromosome number is twelve. We have found this number so frequently that there can be no doubt of it (Pl. 16, figs. 1–4). In the cells which we call spermatogonia, however, mitotic figures show equally clearly six chromosomes. In equatorial plate and anaphase figures the number six is always perfectly clear (Pl. 16, figs. 6, 8, 9), though in a few figures in quite young nymphs (Stage I, first or second day after hatching from the egg) the chromosomes are visibly double with an arrangement of the pairs which does not suggest a longitudinal split preparatory to division (Pl. 16, fig. 7). And in prophases, especially in Stage I nymphs, but also in the later stages, this doubleness of some of the chromosomes is more conspicuous (Pl. 16, fig. 5). In no male germ-cell, from nymphs a few hours old onwards, have we ever found twelve distinct chromosomes such as occur in the somatic cells; the only possible exception is in the rather large single cells mentioned above, and we are confident that these are follicle cells and not cells of the "germ-track." The question therefore arises whether the numerous testicular cells which in the equatorial plate and later stages of mitosis clearly have half the somatic chromosome number, are indeed spermatogonia, or whether they are spermatocytes; and if they are spermatogonia, what is the explanation of their possessing the haploid (reduced) chromosome number? In favour of their being spermatocytes there is, in addition to their number of chromosomes, the fact that after the growth phase there is only one cell division—the unequal division referred to in Section A above. These facts caused us at first to regard these cells as primary spermatocytes, but we have been compelled to conclude that they are really spermatogonia by the fact, which appears to us indubitable, that they divide repeatedly before the growth phase commences. Quite typical mitotic figures with six chromosomes occur in the testis of Stage I nymphs not yet a day old, at a time when the testes are very small and contain comparatively few cells. Several days later, in young Stage II nymphs, the testes are much larger, but there has been no increase in the size of most of the cells, and the number of the cells is greatly increased. The small cells at the apex of these older testes, and on into Stage III, have exactly similar mitotic figures with six chromosomes. It seems evident, therefore, that these cells are true spermatogonia, but that for some reason they contain the haploid chromosome number. On the analogy of the Hymenoptera, we supposed that possibly the male louse developed from an unfertilised egg, and had the haploid number throughout its germ-track, but, as will be seen from the section on the maturation of the egg, this is almost certainly not so. Eggs laid by virgin females never develop, and no evidence was obtained from sections of eggs preserved shortly after being laid that the egg-nucleus ever segmented without conjugating with a sperm nucleus. The only suggestion that we have to offer, therefore, is that in Pediculus there is precocious pairing of the chromosomes, and that the six chromosomes seen in spermatogonial mitoses are each double or bivalent consisting of a pair of univalents united together. If the pairing were not yet quite complete, this would account for the prophase figures with double chromosomes, or sometimes with apparently as many as seven or eight, to which we have already referred.

In this connection we would refer to the work of Wilke (**12**) on the spermatogenesis of Hydrometra. His paper is somewhat obscure, but he writes quite definitely that there are eleven chromosomes in the spermatogonial mitoses, and twelve, which he interprets as ten bivalents and two univalents, in those of the primary spermatocytes. If he is correct, it would appear that in this genus also the spermatogonial chromosomes are bivalent.[1]

The resting spermatogonial nucleus is large in comparison with the small volume of the cytoplasm, and contains a conspicuous nucleolus (Pl. 16, fig. 5). In Stage II (after the first moult) groups of these cells in the proximal and middle part of the testis begin to increase in size, and the mitochondria become visible in the cell body (Pl. 16, figs. 15–17). The description of these, and of the "acroblast," which appears rather later, is postponed to a later section. When the cells have attained their full size the nucleolus disappears, and six chromosomes appear in the nucleus. With Breinl's stain (safranin and methylene blue; for method see 'Ann. Trop. Med. and Parasitology,' vol. i, p. 470) the nucleolus stains blue, suggesting that it consists of chromatin, which is possibly used up in the formation of the chromosomes; we have not followed the process in detail. In the majority of cells at this stage, which must now be called spermatocytes, six chromosomes are visible in the nucleus, differing among themselves somewhat in size, but hardly enough to recognise them as individuals; one is sometimes recognisably smaller than the rest. In some groups of cells, however, it is quite evident that each of these chromosomes is double (Pl. 16, fig. 10), composed of two short rods which may appear paired either end-to-end or side-to-side. The rods are so short in comparison with their width that observations on the mode of pairing can hardly be trustworthy, but it is fairly evident, from the degree of separation of the members of each pair, that the doubleness is due to pairing and not to splitting. The nuclear membrane now disappears, and a spindle is formed between two conspicuous centrosomes lying near the margins of the cell. A striking peculiarity of the spindle at this stage is that it is always very eccentric, it lies at one side of the cell, so that the whole of it is contained in considerably less than one hemisphere (Pl. 16, fig. 11), the other side of the cell being largely occupied by the conspicuous mitochondrial body, which by this time is very compact. The chromosomes became arranged on the spindle in an equatorial plate, and, in some cases at least, the mitosis has reached the early anaphase stage, when the form of the cell undergoes a striking change, which from the comparative rarity of the stages must take place very quickly. The spindle elongates considerably, and one pole of it, with its centrosome, is carried outwards at the tip of a finger-like process, which in the late anaphase projects to a distance of half the diameter of the cell or even more (Pl. 16, figs. 12, 13, 14). Occasionally it is possible to count six chromosomes travelling toward each pole, but in the late anaphase the spindle is extremely narrow, and the chromosomes become so bunched together that it is rarely possible to see them separately, especially in the part of the spindle contained in the narrow process. The six chromosomes at what may be called the proximal end of the spindle (the end directed away from the finger-like process) travel to the pole and form a vesicular nucleus in the neighbourhood of the mitosome and "acroblast," which have remained stationary in the body of the cell. The chromosomes which have entered the finger-like process pass outwards to its tip and there form a compact nucleus or chromatin mass. The protoplasm of the process then seems to round itself off around this nucleus into a little mass resembling a polar body, which at first is connected with the body of the cell by a narrow neck (Pl. 16, fig. 30), but soon becomes completely separate (Pl. 16, figs. 28, 29). These little masses remain for a considerable time in the neighbourhood of the spermatids and around the heads of the developing spermatozoa; they undergo no further development and seem finally to disintegrate.

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[1] In a species of Gerris, a related genus of Hydrometridæ, I find that the spermatogonia contain twenty-four and the spermatocytes twelve chromosomes, which suggests that possibly Wilke is mistaken.—L. D.

As will be seen from Pl. 16, figs. 11–14, the whole division has a close resemblance to the single nuclear division in the spermatocytes of the hive bee, as described by Meves (**10**) and by Mark and Copeland (**8**), or to the first spermatocyte division of Aphids as described by Morgan (**11**) and by von Baehr (**1**). In the case of the Aphids a teleological explanation (if the words may be used) of the unequal division can be given, for the small spermatocyte lacks a sex chromosome which is present in the large one. In the bee, however, no adequate explanation of the phenomena has ever been given, and we are equally at a loss to account for the similar behaviour of the spermatocytes of the louse. And in one respect the louse is still more anomalous, for the bee resembles all other Hymenoptera in having only one spermatocyte division, which is doubtless equational, in correspondence with the fact that its germ-cells have throughout the haploid number of chromosomes, since the male is developed from an unfertilised egg. There is no reason, however, for believing that this is true of the louse, and though the absence of a second spermatocyte division is doubtless correlated with the fact that the spermatogonia have six instead of twelve chromosomes, there is still considerable obscurity as to the cause of there being only one spermatocyte division. For it seems certain that the apparently haploid chromosome number of the spermatogonia is due to precocious pairing of the chromosomes, perhaps comparable with the paired arrangement of homologous chromosomes characteristic of the Diptera (cf. Metz, **9**), and this is confirmed by the existence of conspicuously double chromosomes in the spermatocyte prophases of well-preserved testes (Pl. 16, fig. 10). If, therefore, the general belief is justified that the heterotype division is, so to speak, a special incident, due to the pairing of the chromosomes, intercalated into the middle of an ordinary mitosis which is completed in the succeeding homotype division, it should be expected that a second division would be required in the louse, as in all other known cases of sexually produced organisms, to separate the halves of longitudinally split univalents. In the louse we have found no evidence of the existence of this split, and it would appear to be unique in having a heterotype division which is not followed by a homotype.

Immediately after the spermatocyte division described above, the large cells (as distinguished from the minute polar cells), which must now be called spermatids, begin the final stage of their development—their transformation into spermatozoa. The nucleus has at first a considerable size and contains masses of chromatin, and sometimes traces of a reticulum are visible (Pl. 16, fig. 26), but as development proceeds the nucleus shrinks and the chromatin becomes aggregated into a mass in the centre (Pl. 16, figs. 27–30). The later stages of the nucleus, when the cell is elongating to form the spermatozoon, are somewhat obscure, and will be most conveniently considered in the section on spermiogenesis ("spermateleosis") after the behaviour of the mitochondria, centrosomes and "acroblast" have been described.

C. Cytoplasmic Structures and Later Development of the Spermatozoa.

We have not succeeded in demonstrating mitochondria with any certainty in the spermatogonia. In some cells of this stage small stained granules appear in the neighbourhood of the nucleus, but they are not constantly seen, and we are not certain that when they are present they may not be artefacts. When the growth-phase has begun, however, a cloud of staining granules appears at one side of the nucleus, which is now very excentric and often almost at one margin of the cell (Pl. 16, figs. 15, 16). Almost from its first appearance this mass of granules is vacuolated, giving a foam-like appearance, and a little later the outer vesicles enlarge or coalesce to form prominent vacuoles enclosing a more solid, deeply-staining mass. At this stage the appearance of the mitochrondrial body is that which Gatenby (**3**) ascribes to faulty fixation (Pl. 16, figs. 18–21), and we are not prepared to deny categorically that this may not be the explanation of the appearances seen in our preparations. Before assuming that this is so, however, it must be noticed that several facts point to their being a true representation of the natural condition. Firstly, cells at this stage fixed in F.w.a. $\frac{1}{4}$ (dilute Flemming without acetic) are no less vacuolated than those fixed in normal Flemming. Secondly, in the later stages of the spermatid we have beautiful figures of the mitosome, showing a definite and fairly constant structure which we think can hardly be artificial, and these occur in the same testes which have the vacuolated mitochondrial mass in the spermatocytes. Thirdly, the vacuoles of the early stages appear to play a definite part in giving origin to the structure of the mitosome of the spermatid. Just as Gatenby in the Lepidopteran testes found vesicles coalescing to give rise to his "mitochondrial spireme," so we find spermatocyte vacuoles apparently coalescing to give rise to the structure of the mitosome (Pl. 16, figs. 21–24). We think it not improbable that our mitochondrial figures in the younger cells may have undergone some distortion in fixation, but we hesitate to ascribe the whole structure as we see it simply to the action of the fixative. We find that in some cells preserved in normal Flemming, and in all in Flemming with two or three times the normal amount of acetic acid, the mitochondria are more or less completely dissolved, but we do not find large differences in structure in so far as structure is visible.

As the spermatocyte approaches division, the deeply staining substance of the vacuolated mass of mitochondria becomes aggregated as a more compact mass, surrounded by a few large vacuoles which separate it from the surrounding cytoplasm, and these vacuoles appear to coalesce into two, which enclose a central mass between them (Pl. 16, figs. 20–24). The central mass itself does not appear to be homogeneous, but to contain a space, or sometimes several spaces, enclosing a substance which stains less deeply. In the spermatocyte until after the unequal division the whole body is not far from spherical, consisting of two hemispherical vacuoles enclosing between them a somewhat ovoid, deeply-staining, more or less hollow mass.

Before this stage is reached there has appeared near the nucleus a spherical, deeply-staining body about the size of the nucleolus (Pl. 16, figs. 10, 14, 25–29). Since it appears at a time not far removed from that at which the nucleolus disappears, we thought at first that it might possibly be the nucleolus which had somehow emerged from the nucleus into the cytoplasm. Further examination, however, showed that the extra-nuclear body co-exists for a time with the nucleolus, and sections stained with Breinl show that the nucleolus stains blue, and the extra-nuclear body, which we provisionally call by the name "acroblast," adopted from Gatenby, stains purplish-red. In some spermatocytes the acroblast may be double about the time of its first appearance, but it is always a single spherical body later. It is probably constantly present in suitably preserved cells, but it is not always visible; we suspect that when it cannot be seen it may have been dissolved by reagents. The mitochondrial mass also shows extraordinary variability in staining capacity, even in adjacent cells in the same section, and it seems probable that the visibility or apparent absence of the "acroblast" may be due to the same cause, whatever this may be. We have been unable to determine with certainty either the origin or nature of the "acroblast," but on the analogy of the bodies in Lepidoptera, which Gatenby calls by the same name, we suspect that it belongs to the Golgi apparatus. Attempts to prove this by Kopsch's method (prolonged fixation in osmic acid without staining) have, however, failed to confirm this belief; in sections of testes treated in this way we have failed to find the body. Its subsequent history, so far as we have been able to follow it, will be described below in conjunction with that of the spermatid nucleus.

The chief remaining structure of interest in the developing spermatozoon is the centrosome apparatus and axial filaments. There is nothing peculiar about the centrosomes of the spermatocyte, except the extrusion of one of them with the small "polar" cell in the unequal division. Very shortly after that division, however, when the cell is just beginning to elongate, it may be seen that the centrosome lying between the nucleus and the mitosome is double (Pl. 16, figs. 24, 25).

This stage is difficult to find, for very shortly after the division, from each of the halves of the double centrosome an axial filament begins to grow out (Pl. 16, fig. 26); the two filaments grow back in close connection with the outer sheath of the mitosome, and appear to have thickenings ("distal centrosomes") at their free ends. When they reach the posterior margin of the cell these distal centrosomes usually remain on the margin (Pl. 16, figs. 27, 29, 31); they sometimes appear to be carried out a short distance on thin processes like the antennæ of a butterfly (Pl. 16, fig. 30), but this appearance is not constant. A continuation of the axial filaments grows out beyond them as two thin flagella-like threads, or, probably through their being twisted together, as what looks like one thread (Pl. 16, figs. 29–31).

It can sometimes be seen that one of the axial filaments is thicker than the other, but in consequence of their close proximity to the edge of the mitosome this is not always easy to make out (Pl. 16, fig. 27). Transverse sections of elongating spermatids show very clearly the paired axial filaments running along the mitosome, but completely outside it, and the same is seen in transverse sections of the tails of well-advanced spermatozoa. There can be little doubt that the paired centrosomes and axial filaments are connected with the absence of a second spermatocyte division.

The mitosome in the spermatid has the same general structure as was described above in the mature spermatocyte, except that the whole body now appears bilaterally symmetrical, consisting of two outer symmetrically placed vacuoles enclosing a central, more deeply staining mass. This central mass often contains a cavity or vacuoles; many preparations (e.g. Pl. 16, fig. 32) give the impression that it is divided into two in the plane of junction of the two outer vacuoles, but it is possible that this appearance is due to the line of junction of the outer vacuoles running over its surface. Sometimes the inner mass clearly contains several distinct cavities (Pl. 16, fig. 25). For a time these structures gave us the impression that there was a spirally-coiled plate lying in a non-staining space (cf. Pl. 16, fig. 28), but closer examination leads us to believe that this appearance is due to looking at the body obliquely, and that nothing corresponding with a "mitochondrial spireme" exists in Pediculus. As the cell elongates, the mitosome becomes drawn out in length (Pl. 16, figs. 33–35), and at a rather later stage the greater part of it is contained in a conspicuous swelling towards the posterior end of the long cell (Pl. 16, figs. 35, 36, 48A). After this stage the cells become so attenuated that we do not feel confident in tracing the fate of the mitosome further.

There remains to be described the development of the anterior end of the spermatid—the nucleus and acrosome. In the early spermatid the nucleus lies at what will be the anterior end, the two centrosomes just behind it, and the acroblast is behind or on one side of these (Pl. 16, figs. 33–37). For some time no important change takes place, but when the cell is considerably elongated the acroblast comes into contact with the nucleus, which is now small and spherical, and usually stained evenly and faintly or with a more deeply staining posterior area next to the centrosome. The acroblast now becomes pressed against the nuclear membrane and begins to spread out over one side of it like a small cap, and then grows forward over the nucleus until its anterior end projects in front of it (Pl. 16, figs. 38–41). In successful Breinl preparations at this stage the nucleus is stained blue and the acroblast cap red. Most of the staining substance of the acroblast (as seen in iron-hæmatoxylin preparations, which show the detail much better than those stained in any other way) is concentrated in the forward extension, but there is generally a band of more deeply stained substance running back like a ridge along the middle line of the cap to another mass at the posterior end close to the centrosomes. The whole structure is so small and the parts in such intimate contact that it is difficult to make out with certainty how much is of nuclear origin and how much is derived from the acroblast, but in Breinl preparations of this stage the cap appears red and most of the posterior part blue, which confirms our conclusion, derived from hæmatoxylin preparations which show more detail, that at least the whole of the projecting portion in front of the nucleus is derived from the acroblast. Whether some part of the acroblast remains at the posterior end is less certain, but as Breinl preparations often have a red region just in front of the centrosomes, it seems probable that this is so. The acroblast, however, does not seem to give rise to the deeply stained oval body which lies apparently within the nucleus at the posterior end of more elongated heads (Pl. 16, figs. 42–48); this body does not stain red with Breinl, and we incline to the belief that it is part of the nucleus. At this stage the whole head is becoming narrowed and elongated, and the acroblast projects in front of it as a point, widening behind to enclose the nucleus; it appears to have a shape something like that of the prow of a canoe, or like the cap of an Eschscholtzia bud when it is split along one side as the flower begins to open. There can be no doubt that the anterior projection becomes the acrosome, but as the head elongates the whole structure becomes so narrow and evenly stained—black with hæmatoxylin and uniform bright red with Breinl—that the further differentiation is impossible to follow.

D. Pediculus capitis.

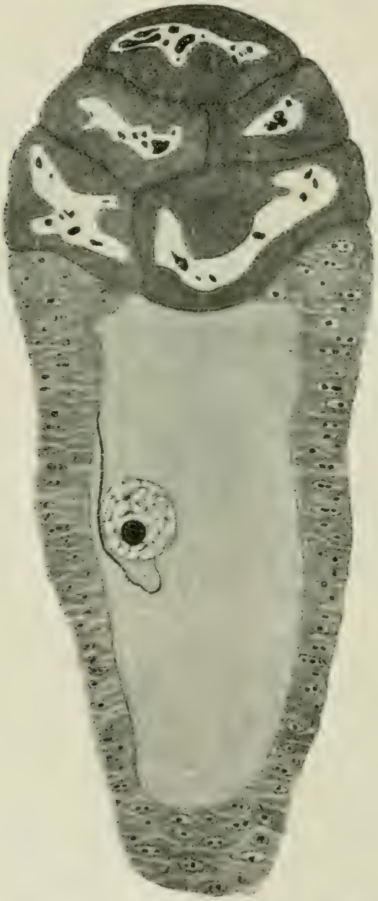
The description given above is taken from our preparations of P. corporis, though a few of the figures with which it is illustrated, especially of the head of the developing spermatozoon, are drawn from sections of P. capitis. We have found no constant difference between the two forms as far as cytology is concerned; as far as we have been able to see, the chromosomes, mitochondrial body, acroblast, centrosomes and axial filaments are alike in both. The capitis sections gave the best figures of developing spermatozoa, but it is probable that this is an accidental effect of technique, and not due to any real distinction. Our work, therefore, as far as it has progressed, has not thrown any light on the cause of the production of the intersexes found in the hybrids bred by Bacot.

4. Observations on the Development, Maturation and Fertilisation of the Egg.

We add here a few observations on oögenesis which are admittedly extremely incomplete, and we give them only to draw attention to several points of interest which we hope others may take up if we are unable to continue the work. We have not succeeded in finding any indubitable oögonial divisions, but equatorial plates of mitoses in follicle cells of the more advanced egg-tubes show twelve chromosomes very clearly. In young oöcytes six threads may be counted in the nucleus in the synapsis stage.

As the egg-tubes develop they become divided into follicles enclosing the growing oöcytes alternating with nutritive chambers. The latter consist of cells with large irregular nuclei which appear to be degenerating; each nutritive chamber is anterior to the oöcyte in connection with it. As the oöcyte enlarges, a deeply staining rod appears in the neighbourhood of the nucleus, and becomes a very conspicuous body in the older oöcytes (Text-fig. 1). It is of considerable length, often somewhat bent, and appears to consist of a hard substance, for in transverse section the portion in the thickness of the section is often displaced to some extent by the razor. Very often it has a small clear space around it, as if it had contracted in fixation and had left a space between it and the yolky cytoplasm of the egg. Sometimes in older oöcytes we have found a shorter accessory rod near the large one, and in young oöcytes we have seen two very small rods apparently shortly after their first appearance, but most commonly only one is present. Although we have spent considerable time in searching for its origin, we have not yet got any clue as to how the rod arises, and its fate is no less obscure. The egg in the oviduct becomes enclosed in a hard shell, which cannot be cut with a microtome, and in eggs immediately after they are laid, from which it is not difficult to peel off the shell, we have failed to find the rod. We believe that the nature and origin of this rod would well repay fuller investigation.

TEXT-FIG. 1.



Growing oöcyte of *Pediculus corporis* enclosed in its follicle; nutritive cells at the anterior end. The deeply staining rod is seen close to the nucleus.

A no less enigmatic structure is present in the egg at the time of laying. At its posterior end there is a mass of irregularly coiled bodies, which stain rather deeply with hæmatoxylin, lying at the edge of the yolk. Freshly laid eggs, even from the same female, differ widely from one another in the thickness of the layer of finely granular protoplasm which surrounds the inner yolky mass. In some eggs this layer is quite thick, in others very thin, and there is similar variation in the size and position of the "posterior mass," as we call for convenience this group of coiled granular substance. In eggs with a thick outer layer the posterior mass usually lies in it and does not project into the yolk, but when the outer layer is thin, it is usually invaginated into the central yolky substance as a sort of vesicle, filled with the "posterior mass." We have not attempted to follow the development of the egg beyond the early segmentation stages, so we can say nothing about the fate of the posterior mass; we understand that work on the subject is in progress in other hands. We would only point out that in appearance the mass has a very close resemblance to the mass of supposed symbiotes figured by Buchner in the egg of certain Hemiptera (**2**).

We have, however, attempted to trace its origin, but, as in the case of the rod mentioned above, have not yet succeeded in doing so. The mass somewhat resembles the degenerating nuclei of the nutritive chamber of the ovarian oöcyte, and we suspect that it may be derived from it. The difficulty of this suggestion is that the nutritive chamber is at the anterior pole of the egg, at the opposite end from the "posterior mass." Until we have made satisfactory preparations of the full-grown oöcyte in the oviduct, in which we have not yet been successful, the matter must be left in suspense.

It is by no means easy to obtain satisfactory preparations of the maturation-divisions of the egg of Pediculus. The shell is extremely tough and resistant, and though it can be removed from eggs preserved in Gilson's or Carnoy's fixatives and subsequently placed in alcohol, we have not succeeded in shelling unfixed eggs, and in eggs preserved in their shells the fixation is not very good. Gilson's solution gives much better results than anything else we have tried. We find that in order to remove the shell it is necessary that the egg should be treated with solutions containing chloroform, but it is possible to use acetic alcohol or acetic alcohol sublimate without chloroform as a fixative, and to treat the egg with a mixture of alcohol and chloroform afterwards, in order to loosen the shell.

Although we have cut sections of a large number of eggs preserved within an hour or two hours after they were laid, we have found no case of the actual polar mitotic figures. Some eggs appear to begin their development in the oviduct, and to be already segmenting when they are laid, but others are laid before the conjugation of egg and sperm nuclei takes place. We have obtained several figures showing two or three polar nuclei near the edge of the egg (the outer polar nucleus is very small and apparently soon degenerates), and some of these eggs show either both the egg-nucleus and sperm-nucleus, sometimes with the tail of the spermatozoon some distance away, or if the egg is a little older, the first or second segmentation divisions may be found. In later eggs the polar nuclei have disappeared. A curious feature of the later segmentation divisions is that many of the nuclei appear to be double, or sometimes triple—that is to say they often consist of two or sometimes three vesicles in contact, each containing a nuclear reticulum. We have found no evidence that the egg may ever develop without conjugation of egg- and sperm-nuclei.

5. BREEDING EXPERIMENTS.

Since Hindle (**6**, **6a**) found that the offspring of a large proportion of individual pairs of lice were either all male or all female, we hoped that by fixing the eggs of such pairs during the maturation stages we might obtain evidence with regard to the cytological basis of sex-determination in the louse. We therefore isolated a number of virgin females and paired them each with a single male. Altogether eighteen such pairings were made, in addition to several individuals which were paired with a second mate after the first mate had died.

The original pairings are lettered A–S in the following table, and when the female of a pair was used again with a fresh mate, the second pair is represented with the same letter with a subscript 2, thus C_2. The number of eggs laid was nearly always counted, and sometimes they were reared in separate successive batches as in mating J, to see whether the sex-ratio varied between the earlier and later eggs. The sex of the offspring was determined by dissection, usually in Stage II. We have to record our indebtedness to Dr. Keilin for kindly doing some of these dissections while one of us was away from Cambridge and the other temporarily prevented by indisposition from taking charge of the experiments.

DATE OF MATINGS.

Pair.	Number of eggs.	Offspring and sex.	
A	12	6 ♂ 6 ♀	
B	49	28 ♂ 20 ♀	
B_2 ♀ of B, fresh ♂ . .	19	12 ♂ 12 ♀	
C	49	27 ♂ 18 ♀	
C_2 ♀ of C, fresh ♂ . .	20	13 ♂ 4 ♀	
D	Infertile		
D_2 ♀ of D, fresh ♂ . .	24	22 ♂ 1 ♀	
E	Infertile		
E_2	10	— 3 ♀	
F	40	24 ♂ 15 ♀	
G=fresh ♀ × ♂ used in A .	48	23 ♂ 21 ♀	
G_2 ♀ of G, fresh ♂ . .	15	6 ♂ 5 ♀	
H	Infertile		
J first batch . . .	19	13 ♂ 5 ♀	Total: 31 ♂ 40 ♀
second batch . . .	Not counted	6 ♂ 23 ♀	
third batch . . .	„	12 ♂ 12 ♀	
K	64	♂ ♀	not counted
L	28	13 ♂ 4 ♀	
M	14	5 ♂ 6 ♀	
N	39	♂ ♀	not counted
O	28	♂ ♀	„
P	Not counted	5 ♂ 10 ♀	
Q	30	♂ ♀	not counted
R	14	♂ ♀	„
S	18	♂ ♀	„

As will be seen from the table, there was no mating which certainly gave offspring of one sex only. B_2 gave 12 ♂ and one female recorded as doubtful out of 19 eggs, and D_2 22 ♂ and 1 ♀ out of 24 eggs. The family E_2, which contained only three females, is not large enough to be significant. The fact that in mating J there was such large variation in sex-ratio in different batches (in batch 2, 14 females were dissected in succession and then a group of males found), suggests that possibly the existence of apparently unisexual broods may be due rather to chance fluctuations than to a physiological difference in the parents. Hindle kept no record of the number of eggs laid, so did not know the mortality rate, but in our experiments in most families the sex of nearly all the eggs was recorded, except in the last few matings, in which the conduct of the experiments was accidentally interfered with.

6. Summary.

The main facts of the spermatogenesis have been given in Section 3 A above. The most important points are as follows:

(1) The somatic chromosome number of both sexes is twelve, but spermatogonial mitotic figures show only six. There is some evidence that these are double, and we ascribe the existence of the apparently haploid number in the spermatogonia to premature pairing.

(2) There is only one spermatocyte division, which is extremely unequal, leading to the separation of a minute polar-body-like cell which degenerates.

(3) The centrosome of the spermatid is double, and from each half an axial filament grows out, so that the developing spermatozoa have two conspicuous axial filaments.

(4) The development of the mitochondrial body is described.

(5) The acrosome is derived from a deeply staining body which appears in the spermatocyte and becomes applied to the nucleus of the spermatid like a cap.

(6) The existence of a deeply stained rod, of unknown origin and fate, in the growing oöcyte, and of a posterior

mass of stained granules in the mature egg, is shortly referred to. The egg nucleus undergoes two polar divisions, and fertilisation appears to be essential if the egg is to develop.

(7) Breeding experiments did not confirm Hindle's observation that broods consisting only of males or only of females are frequent. Some broods with great preponderance of one or the other sex were obtained.

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EXPLANATION OF PLATE 16,

Illustrating Prof. L. Doncaster's and Mr. H. G. Cannon's paper on "The Spermatogenesis of the Louse (Pediculus corporis and P. capitis), with some Observations on the Maturation of the Egg."

Description of Figures.

[The figures are from freehand drawings, made with Zeiss apochromatic N.A. 140, 3 mm. or 2 mm. objectives, and compensating ocular 12. They are not all exactly to the same scale; in most of the figures the magnification is about 3000 diameters; in figs. 1–4, 6–9, 38–50 it is about twice as great. All figures are from preparations of P. corporis except when it is stated in the description that the figure is from P. capitis. All figures except Nos. 7, 8, 15, 16, 17, 25 are from drawings by H. G. Cannon.]

Figs. 1–14 illustrate chiefly the chromosomes in spermatogonia and spermatocytes.

Fig. 1.—Equatorial plate from follicle cell of Stage II testis; twelve chromosomes. (Flemming, acetic × 2.)

Fig. 2.—Equatorial plate from body-cell of Stage II female; twelve chromosomes. (Flemming.)

Fig. 3 (P. capitis).—Equatorial plate from follicle cell of Stage II testis; twelve chromosomes. (Flemming.)

Fig. 4 (P. capitis).—Equatorial plate from body-cell of Stage III female; twelve chromosomes. (Flemming.)

Fig. 5.—Spermatogonial prophase, Stage III; chromosomes partially double and nucleolus. (Flemming.)

Fig. 6.—Typical spermatogonial equatorial plate, Stage III; six chromosomes. (Flemming.)

Fig. 7.—Spermatogonial equatorial plate from young Stage I nymph; six chromosomes, all double. (Flemming.)

Fig. 8.—Spermatogonial equatorial plate from young Stage I nymph; six chromosomes. (Flemming.)

Fig. 9 (P. capitis).—Spermatogonial equatorial plate from Stage II male; six chromosomes. (Flemming.)

Fig. 10.—Prophase of spermatocyte division, Stage II male; six double chromosomes; acroblast near the mitochondrial body. (Flemming, acetic × 2.)

Fig. 11.—Metaphase of spermatocyte division, Stage III male; spindle at one side of the cell. (Flemming.)

Fig. 12.—Early anaphase of spermatocyte division, Stage III male; the finger-shaped process has hardly begun to appear; almost certainly six chromosomes at each pole. (Flemming.)

Fig. 13 (P. capitis).—Later anaphase of spermatocyte division, Stage II male; appearance of finger-shaped process; six chromosomes are clearly visible in the body of the cell; the pair nearest the centrosome in the process are not quite certainly separate. (Flemming.)

[Note.—The acroblast and mitochondrial body are not preserved in figs. 11–13.]

Fig. 14.—Late anaphase of spermatocyte division, Stage II male; the process has become long and narrow and the chromosomes are aggregated in its tip; the large cell retains the mitochondrial body and acroblast. (Flemming.)

[Note.—The complete separation of the small cell at the close of the spermatocyte division is shown in figs. 28–30.]

Figs. 15–32 illustrate the development of the mitochondrial body, acroblast and axial filaments in the spermatocyte and young spermatid.

Fig. 15.—Young spermatocyte with first appearance of mitochondria. Stage II male. (F.w.a. ¼.)

Fig. 16.—Older spermatocyte: mitochondrial mass showing vacuolation. Stage II male. (F.w.a. ¼.)

Fig. 17.—Older spermatocyte: mitochondrial mass much vacuolated. (F.w.a. followed by potassium bichromate.)

Figs 18–21.—Coalescence of mitochondrial vesicles into large vacuoles: the appearance represented in fig. 20 is that most frequently seen. Fig. 18, from section preserved in Flemming; figs. 19–21, from smear preparations.

Figs. 22, 23.—Coalescence of vacuoles to form two large vacuoles enclosing a stained mitochondrial mass. (Smear preparations. F.w.a.)

Fig. 24.—Young spermatid, with acroblast next to the nucleus, double centrosome, and typical mitosome of simple structure. (Flemming.)

Fig. 25.—Similar stage, from the same individual, with mitosome of more complex structure. (Flemming).

Fig. 26.—Beginning of outgrowth of axial filaments: mitosome in optical section. (Flemming.)

Fig. 27.—Further growth of axial filaments, with "distal centrosome" at posterior edge of cell. Mitosome seen from the side, so that its bilateral structure is not visible. (F.w.a. ¼.)

Fig. 28.—Axial filaments beginning to protrude from cell; mitosome seen obliquely. The small cell separated at the unequal spermatocyte division is near the anterior end of the cell. (F.w.a. $\frac{1}{4}$.)

Fig. 29.—Similar cell from same testis, with further growth of axial filaments. (F.w.a. $\frac{1}{4}$.)

Fig. 30.—Similar cell from same testis; axial filaments united outside cell (probably intertwined), and distal centrosomes at the end of short processes. This condition is not general, and is possibly due to artefact. Small "polar" cell still attached to spermatid. (F.w.a. $\frac{1}{4}$.)

Fig. 31.—Beginning of elongation of spermatid; same testis as figs. 28–30. (F.w.a. $\frac{1}{4}$.)

Fig. 32.—Transverse section of same stage as last, from same testis, showing two axial filaments cut across just outside the mitosome, the structure of which is well shown. The position of the nucleus, at a different focus, is indicated.

Figs. 33–50 illustrate the conversion of the spermatid into the spermatozoon.

Fig. 33.—Elongation of the spermatid; mitosome still vacuolated. (F.w.a. $\frac{1}{4}$.)

Figs. 34–37.—Continued elongation, with development of a swelling at the posterior end, into which the greater part of the mitosome becomes concentrated. The structure of the mitosome is no longer distinguishable. (F.w.a. $\frac{1}{4}$.)

Fig. 37A is an oblique section of the head at the stage of fig. 37, showing acroblast, two centrosomes and nucleus.

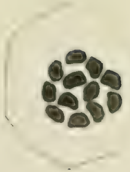
Figs. 38–50 illustrate the development of the head of the spermatozoon.

Figs. 38–42 (*P. capitis*).—Application of acroblast to the nucleus (figs. 38, 39), its spreading forward like a cap (fig. 40) until it forms a projection in front of the nucleus (fig. 41, face view, fig. 41A side-view). Fig. 42 shows the deeply staining body at the posterior side of the nucleus, apparently connected with the acrosome by a band. (Flemming.)

Figs. 43–49.—Elongation of the head and formation of the projecting acrosome from the anterior portion of the acroblast. The stained body at the posterior end of the nucleus is shown especially in figs. 45–47, and figs. 45A and B represent optical transverse sections (A) through it, and (B) in front of it. (All F.w.a. $\frac{1}{4}$.)

Fig. 48A represents a complete spermatozoon at the stage of fig. 48, from a smear preparation. (Smaller scale.)

Fig. 50.—The head of a nearly mature spermatozoon; smear preparation.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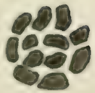
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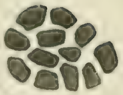
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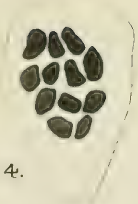
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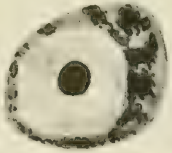
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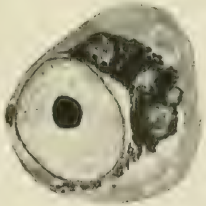
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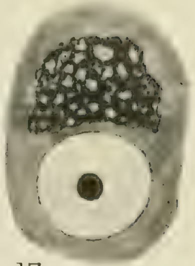
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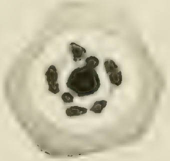
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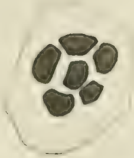
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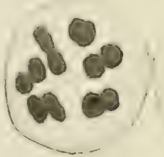
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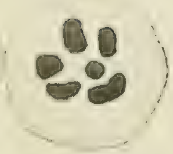
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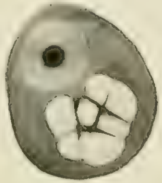
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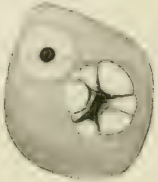
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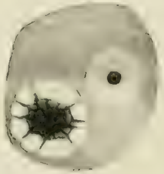
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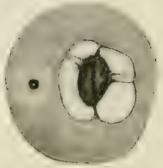
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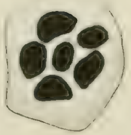
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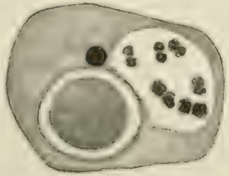
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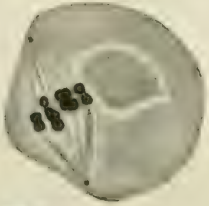
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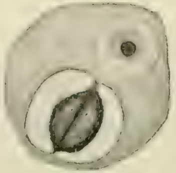
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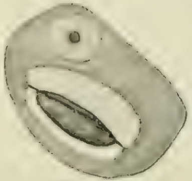
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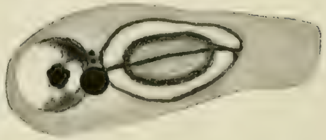
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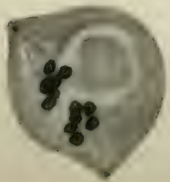

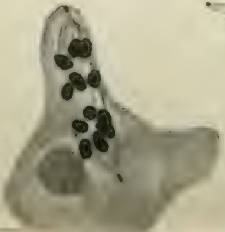

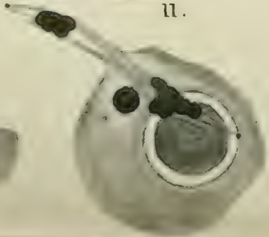

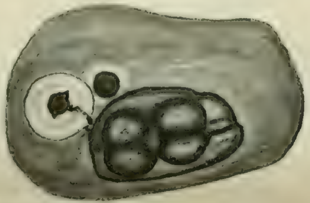

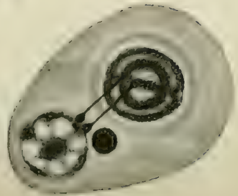
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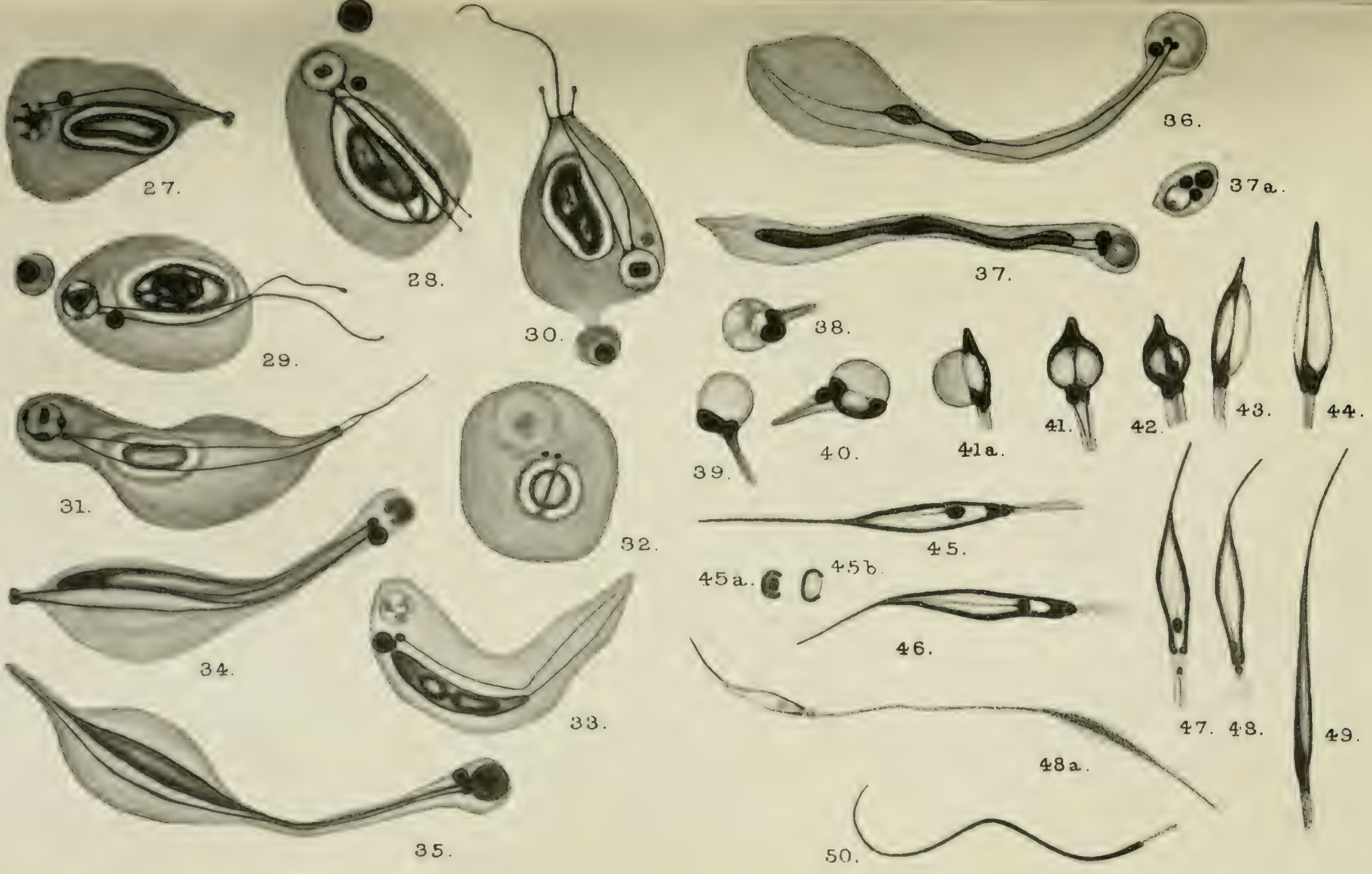

SPERMATOGENESIS OF LOUSE.

Huth, London.

Observations on an Intra-nucleolar Body in Columnar Epithelium Cells of the Intestine.

By

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With Plate 17 and 1 Text-figure.

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1. INTRODUCTORY.

IN the course of observations on the columnar epithelium cells of the cat's intestine, it was noticed that the Cajal formol-silver nitrate method for the Golgi apparatus sometimes demonstrated one or more intra-nucleolar granules. In this paper an attempt has been made to describe these bodies in the resting cell and their behaviour during mitosis.

The term "nucleolus" is somewhat indiscriminately applied to one or more bodies, usually rounded or oval, lying within the nucleus. When these structures are basophil in staining reaction they are termed "karyosomes" or "chromatin nucleoli"; when oxyphil, "plasmosomes" or sometimes "true nucleoli." Throughout this paper the term "nucleolus" is used as a general term comprising both karyosome and plasmosome, there being, so far as I am aware, no other word available—unless a new term were created, which does not seem desirable.

I am indebted to Mr. E. S. Goodrich for advice and criticism. I am also glad to take this opportunity for thanking him for his many kindnesses to me when a student. To Prof. C. S. Sherrington my warmest thanks are due for his interest in my work, while to my friend, Mr. J. Bronté Gatenby, formerly of this Department, I am indebted for training in cytological technique. Finally, part of the work was done in the Zoology Laboratory of University College, London, for which I have to thank Prof. J. P. Hill.

2. PREVIOUS WORK.

In the abundant literature upon the nucleoli of animal cells, many references may be found to intra-nucleolar inclusions, especially in the nucleoli of oöcytes.

Some authors have observed intra-nucleolar granules in fixed and stained material (**3**, **8**), while others have described vacuoles (**6**, **11**). Finally, a few observations have been made upon the nucleoli of cells examined intra-vitam (**1**, **2**). Montgomery, in a remarkable paper (**11**) upon the nucleolus, gives a chronological summary of the literature containing references to intra-nucleolar bodies and vacuoles. Unfortunately most of the observations on these have been made either upon fixed and stained tissues, or on cells examined intra-vitam. Many of the observations were made on material fixed and stained by the crudest methods. It is therefore difficult to distinguish between what may have been artefact and what was not.

The following references will illustrate the scope and nature of the observations hitherto made upon nucleoli:

Haeckel (**17**) states: "Endlich findet man in vielen Eiern (aber nicht in allen) innerhalb dieses Keimfleckes noch ein innerstes Pünktchen, einen Nucleolinus, welchen man Keimpunkt (Punctum germinativum) nennen kann."

Montgomery (**11**), summarising his personal observations, says: "In agreement with the majority of observers, I can attach no particular morphological significance to the nucleolinus; it appears to be only a detached portion of the nucleolar ground substance, to be in most cases absent, and when present to vary greatly in regard to size, position and number. It is undoubtedly the case that many structures which have been described as nucleolini are in reality minute vacuoles, which from their refrangibility appear to be granules. . . ."

Maziarski (**10**), in an interesting study of the nucleus of the intestinal epithelial cells in Isopod Crustacea, notes that plasmosomes frequently contain basophil granules which he homologises with the chromatin.

Prenant, Bouin and Maillard (**12**) say of the nucleolus: "Au lieu d'être homogène, comme c'est le cas habituel, il offre parfois une structure. . . . Bien d'autres complications structurales ont été signalées dans certains nucléoles; une membrane d'enveloppe, etc."

No observations, so far as I am aware, have been made upon nucleoli in material treated by the Cajal method for the Golgi apparatus, with the exception of those of P. del Rio Hortega (**9**). This worker has noted that in mammalian ova "la tâche germinative ou nucléole ovulaire apparait par les méthodes de l'urane comme constituée par des granulations argentophiles disposées périphériquement au nombre de quatre à six ou par petits bâtonnets alignés dans la direction transversale; le nucléole forme une espèce de petite vessie claire d'où se détachent vigoureusement ces granules." In some of the figures in Cajal's memoir (**4**) on the Golgi apparatus intra-nucleolar bodies are depicted, but no reference is made to them.[1] Further, I am not acquainted with any observations on intra-nucleolar inclusions in intestinal epithelial cells of vertebrates either by the Cajal or any other method.

Throughout this paper the intra-nucleolar body studied has been termed a nucleolinus. There are at least eleven terms which have been applied to intra-nucleolar inclusions. Out of these I have chosen the word "nucleolinus," such a course seeming wiser than to burden cytological literature with yet another new term.

3. Material and Methods.

It was found that the formol-silver nitrate method of Cajal (**4**) sometimes demonstrated the nucleolinus of the columnar epithelial cells of the cat's small intestine with extraordinary clearness. In such preparations the nucleolinus was the only impregnated structure within the nucleus.

As counterstains hæmatoxylin and eosin (used according to Scott's method (**13**)), Mann's methyl blue-eosin, pyronin-methyl green, and toluidin blue-eosin were used. For the study of the behaviour of the nucleolinus during mitosis, safranin gave the most valuable results, as its clearness of definition was found to be less affected by the silvering process than was the case with other stains.

In the cat the best preparations were obtained by using the Cajal method unmodified, the technique of which I have described elsewhere (**5**).

In the case of the frog, however, it was not found possible to demonstrate the nucleolinus by the standard Cajal method. After many attempts with various modifications of the original the following technique gave almost invariably successful results:

---

[1] Since the above was written, Cajal's "Manual de Histología Normal" has come into my hands. In this very remarkable text-book, which those who regard mitochondria and Golgi apparatus as artefacts would do well to study, Cajal refers to nucleolini as being frequently artefacts. He adds, however, that within the nucleoli of nerve cells "se observan claramente espérulas interiores y una materia intersticial." [p. 201.]

(1) Fixation for ten to eleven hours in the uranium nitrate-formol mixture made up thus:

Uranium nitrate . . .	2 grm.
Formol (40 per cent.) . .	6 c.c.
Aq. dest.	100 ,,

(2) Rinsing the pieces of tissue in aq. dest. for a few minutes.

(3) Impregnation with a 3 per cent. solution of silver nitrate for thirty-six hours.

(4) Rinsing in aq. dest.

(5) Reduction in the standard solution for one to one and a-half hours.

The material was then washed in water, dehydrated in ascending grades of alcohol, cleared with xylol, and imbedded in wax in the usual manner.

As may be seen by comparison with the ordinary Cajal method (**4**, **5**), the above technique differs from it in (A) the greater uranium nitrate content, 2 grm. instead of 1, and (B) the strength of the silver nitrate-solution—3 per cent. instead of 1·5 per cent. For reasons which I am unable to explain, the action of this modified technique for the nucleolini of the columnar cells of the intestine of the frog is such that these elements are generally the only impregnated structures within the cells, the Golgi apparatus being only very rarely demonstrated.

In addition to the original and modified Cajal methods, control observations on the nucleolini were made by ordinary cytological technique. Teased intestinal mucosa was also examined intra-vitam and with intra-vitam stains.

4. The Nucleolinus in the Resting-Cell.

(A) In the Cat.—In a successful Cajal preparation of the intestinal epithelium the following structures may be seen:

(i) The Golgi apparatus, appearing as a coarse, irregular reticulum, impregnated black, the interspaces of the network clearer than the surrounding ground cytoplasm.

(ii) The mitochondria, impregnated golden to dark-brown. It is a curious fact that only the mitochondria in the outer portion of the cells (i. e. between the nucleus and the striated border) are demonstrated, the mitochondrial group lying between the nucleus and the base of the cells being never shown by this method, although Champy-iron hæmatoxylin preparations demonstrate its presence. That this curious selectivity is not due to the silver impregnation failing to penetrate the whole depth of the cells, is shown by the fact that this differentiation holds good for all the columnar cells throughout the thickness of the intestinal mucosa. It can probably be ascribed to chemical differences in the two mitochondrial groups.

(iii) The nucleolini, impregnated black, and sharply differentiated from the surrounding nucleolar substance.

Reference to Pl. 17, fig. 1, will make clear the various impregnated elements within the cell.

Cajal preparations counterstained with safranin show the nucleolini black, the nucleoli and chromatin pink. The nucleoli number from one to three—most generally two per nucleus. Each nucleolus may contain as many as five nucleolini, but the vast majority have but one or two. The intra-nucleolar bodies are usually round, more rarely stumpy rodlets, measuring approximately 0·2 to 0·5 μ in diameter. Reference to the nuclei drawn in Pl. 17, figs. 2 to 5, will illustrate this.

Now sections of Cajal material stained with pyronin-methyl green show the greater number of the nucleoli to be karyosomes, i. e. they stain with the methyl-green component like chromatin. A few of the nucleoli, however, stain more red than green, thereby approximating the staining reaction of plasmosomes. Observations on the same material counterstained with hæmatoxylin and eosin, used according to Scott's method (**13**), and Mann's methyl blue-eosin, give similar pictures of a gradation from basophility to oxyphility in some of the nucleoli. Variations such as these have been noted within a single crypt.

Occasionally a small, deeply-impregnated granule (see Pl. 17, fig. 2), may be seen inside the nucleus, within the chromatin reticulum, often in close proximity to the nucleolus. Its nature I do not know; possibly it represents the nucleolinus of a degenerated nucleolus or an extruded nucleolinus.

The fact that several nucleolini are often found within a single nucleolus suggests that these bodies can undergo division. Often two may be seen lying in the closest proximity, while sometimes the nucleolinus appears as a dumb-bell-shaped body. In the cat, however, these intranucleolar inclusions are so minute that observations on their division are very difficult.

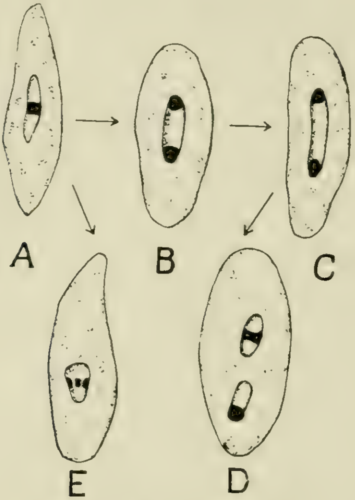
In addition to the Cajal technique, control observations were made after fixation in Champy's fluid and staining in Heidenhain's iron-hæmatoxylin. It was found that after prolonged differentiation bodies within the nucleoli, exactly similar in size, etc., to those impregnated by the silver method, could be demonstrated. Owing to their affinity for the hæmatein being almost equal to that of the surrounding nucleolar material their differentiation is no easy matter, and is best controlled with a water-immersion lens.

(B) The Nucleolinus in the Frog (Rana temporaria).—The body was demonstrated by the modified Cajal technique described above. The nucleolinus is larger than in the cat, being approximately 1 to 1·5 μ in diameter. Its shape also is more variable. Rarely are there more than two nucleolini per nucleolus, while the latter usually number one or two in each nucleus.

The nucleoli of the columnar cells of the frog's intestine are plasmosomes, as is evidenced by their oxyphility after staining with hæmatoxylin and eosin, Mann's methyl blue-eosin, and pyronin-methyl green. In the nucleoli depicted in Pl. 17, figs. 8 and 9, the nucleoli were bright red, the nucleolini black, and the chromatin green. In shape the nucleoli are generally round or oval (Pl. 17, figs. 6 to 9).

There seems but little doubt that the nucleoli are capable of multiplication. The most usual mode is apparently by transverse fission, the nucleolus elongating until it is a rodlet with rounded ends, and then dividing transversely. That the nucleolinus participates in the fission of the nucleolus is very probable; it may often be seen to have a median constriction; often there are two nucleolini lying in the closest proximity within the nucleolus; or they may be situated one towards each end of it. These stages may be easily observed in impregnated material counterstained with safranin. There does not appear to be any fixed stage in the process of nucleolar division at which fission of the nucleolinus takes place. The latter, in fact, frequently divides into two within

TEXT-FIG.



A to D show the usual mode of multiplication of the nucleolus and the nucleolinus. The former elongates and finally divides transversely. The nucleolinus also divides, a portion of it being distributed to each of the daughter-nucleoli. Fig. E shows division of the nucleolinus unaccompanied by that of the nucleolus. Fragmentation of this sort is relatively rare. All the figures represent nuclei of columnar cells from the intestine of the frog. The nucleolini are black. Magnification circa 2690 diameters.

a nucleolus which is apparently unprepared for multiplication. But nevertheless there does seem to be a continuity of the substance of the nucleolinus during the division of the nucleolus.

The fact that in well-impregnated pieces of epithelium the nucleoli, when they number two per nucleus, always contain each a nucleolinus, is strongly suggestive of the continuity of these bodies in daughter-nucleoli.

Reference to the Text-figure will, I hope, make this clear.

Control observations, after fixation in Bouin's or Champy's fluid and staining in toluidin blue-eosin after the former, and iron-hæmatoxylin after the latter, demonstrate the nucleolinus (see Pl. 17, fig. 14).

In addition, the nucleoli were studied intra-vitam and with intra-vitam stains; of these pyronin of 1 in 1000 in frog Ringer stained the nucleolus very sharply. But the latter always appeared to be perfectly homogeneous with all methods.

Examination with the dark-ground illumination also failed to demonstrate the nucleolinus. The probable reason for the failure of intra-vitam observations to demonstrate the nucleolinus is that the refractive index of this body is the same as that of the nucleolus. There are, of course, other instances of intra-cellular structures rarely visible intra-vitam, e.g. the Golgi apparatus.

5. The Nucleolinus during Mitosis.

The study of the behaviour of the nucleolinus during cell-division was restricted to the intestinal epithelium of the frog. For here the nucleolinus is comparatively large, while its impregnation is less capricious and more selective than in the case of the cat. The last is an important point, for in the majority of the intestinal cells examined (including all the mitotic figures observed) the nucleolini were the only impregnated elements within the cells. There could thus be no question of the nucleolini being confused with Golgi apparatus or mitochondrial elements.

In the early prophases of mitosis the nucleolus disappears in so far as can be ascertained by ordinary cytological technique. Nor does it become again apparent until the separation of the daughter-cells is complete.

Now the study of Cajal material, treated by the modified technique and counterstained with safranin, demonstrates the behaviour of the nucleolinus during cell-division. In the early prophase of mitosis, as drawn in Pl. 17, fig. 10, the spireme is beginning to undergo segmentation. There are two dumb-bell-like nucleolini, which, judging by their shape, are about to divide. Fig. 11 depicts a somewhat oblique section across an equatorial plate. The chromosomes are as yet undivided, but the bodies now form two pairs. The stage represented is a late prophase. Fig. 12 shows an early anaphase; the arrows indicate the direction of mitosis, for the section is somewhat oblique. The two chromosome groups, each with a pair of nucleolini, are nevertheless clearly visible. In fig. 15 a late anaphase is depicted, the two groups of daughter-chromosomes are far apart, the interzonal fibres being faintly visible between them. Each group contains two nucleolini, the upper right-hand one appearing to be about to divide. Fig. 16 shows a still later (telophase) stage. The nuclear membrane has re-formed, the chromatin is beginning to become differentiated into isolated condensations, while the nucleolini are clearly visible as a pair of granules in each daughter-nucleus. In fig. 13, which represents a transverse section of the equatorial plate of a prophase, it will be seen that there are only two nucleolini. I have observed this in other prophases, and have come to the conclusion that in all probability the division of the nucleolini often may not occur until the later stages of the prophase. The presence of two nucleolini in the prophase stage is strongly suggestive of the derivation of each from a separate nucleolus, for the majority of nuclei of resting cells contain two of these bodies.

It must be noted that in the original preparations the nucleolini are impregnated black in a perfectly specific manner, while the chromosomes are red and the cytoplasm pinkish—colour contrasts impossible to indicate in pen-and-ink drawings. Nor has any attempt been made to count and depict the exact chromosome number, as, apart from the fact that this would be without any direct bearing on these observations, the safranin does not give a sufficiently precise stain to enable counts to be made on material which has been previously subjected to the Cajal technique.

A comparison of the size of the nucleolini in resting and dividing cells shows that the former become distinctly smaller during mitosis. It may be added that the nucleolinus was observed in both recently metamorphosed and fully grown frogs.

6. Discussion.

It is, of course, impossible to make sweeping generalisations on the nature of the nucleolinus from observations on it in only two different species of animals.

Nevertheless, the fact that such a body can be demonstrated by the Cajal method indicates that it must be of a different chemical nature to the surrounding nucleolar substance, since it possesses, under certain conditions, a specific affinity for silver not shared by the other nuclear constituents.

Furthermore, that the nucleolinus should be present in both karyosomes (in the cat's intestine) and in plasmosomes (in that of the frog) shows that this body is not peculiar to one of these types of nucleoli.

From the behaviour of the nucleolinus during mitosis I believe that there is undoubted continuity of its substance from one cell generation to another in the case of the columnar cells of the frog's intestine. This, of course, is in marked contrast with the fate of the plasmosome with which it is associated, the latter body apparently disappearing during cell division. Montgomery (**11**), with regard to this, says: "There is no substantial basis for Zimmermann's conclusion 'omnis nucleolus e nucleolo,' or more strictly speaking, that the nucleolus in most cases is not derived from a previously existing one." By the term "nucleolus," be it noted, Montgomery means a plasmosome.

As to the significance of the nucleolinus, it is possible that this body initiates the formation of the plasmosome around itself at the completion of mitosis. If this be so, the nucleolinus would be, so to speak, the precursor of the nucleolus. In this case Zimmermann's statement (**14**), "omnis nucleolus e nucleolo," would be true, in so far as continuity of a specialised portion of it—the nucleolinus—was concerned.

The only intra-nucleolar bodies which can, with any degree of certainty, be homologised with the structures described in this paper, are the minute granules or rodlets discovered by P. Del Rio Hortega (**9**) in the nucleoli of ovarian oöcytes (see p. 381). This observer used the same technique as myself.

7. Summary.

(1) The presence of one or more intra-nucleolar bodies in the columnar cells of the intestinal epithelium in the frog and cat has been demonstrated. To these inclusions the term "nucleolinus," originally introduced by Haeckel, has been applied.

(2) By a modification of the Cajal technique for the Golgi apparatus, the nucleolinus has, in the frog's intestine, been impregnated in a perfectly specific manner.

(3) The presence of nucleolini both in karyosomes (e.g. cat) and in plasmosomes (e.g. frog) in the columnar intestinal cells shows that they are not restricted to one of these types of nucleolus.

(4) Evidence has been adduced showing that division of the nucleolus (plasmosomes) in the resting cell is in all probability accompanied by fission of the nucleolinus.

(5) During mitosis the nucleolinus has been shown apparently to divide, a portion or portions of it being distributed to each of the daughter-nuclei. This is in contrast to the behaviour of the nucleolus (plasmosome), which disintegrates during cell division.

(6) The presence of the nucleolinus has been confirmed in the columnar cells of the intestinal mucosa in both the frog and the cat by other and simpler methods than the Cajal technique.

(7) The significance of the apparent continuity of the nucleolinus has been very briefly discussed.

October, 1919.

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EXPLANATION OF PLATE 17.

Illustrating Mr. H. M. Carleton's paper on "Observations on an Intra-nucleolar Body in Columnar Epithelium Cells of the Intestine."

CR. Chromatin reticulum. *CS.* Chromosomes. *GA.* Golgi apparatus. *IF.* Interzonal fibres. *MI.* Mitochondria. *KY.* Karyosome. *PL.* Plasmosome. *SB.* Striated border of columnar cells. *SP.* Spireme. *X.* Nucleolinus.

[All the figures were outlined with camera lucida, paper at table level, with Koritska $\frac{1}{12}$th oil immersion and No. 12 compensating eye-piece. Present magnification of fig. 1 is 1720 diameters, of figs. 2–16 3580 diameters approximately.]

Fig. 1.—Columnar epithelium cells from duodenum of cat, showing Golgi apparatus, mitochondria and nucleolinus. Cajal-safranin.

Figs. 2 and 3.—Nuclei from same material, showing nucleolini within the karyosome. Cajal-pyronin-methyl green technique. In the original preparation the nucleolini are black, the karyosomes and the chromatin network green.

Figs. 4 and 5.—Same as figs. 2 and 3. These figures show the variations in the number of nucleolini, etc. Cajal-safranin.

Figs. 6 and 7.—Nuclei from intestinal epithelium of frog. Modified Cajal-safranin.

Figs. 8 and 9.—From intestinal epithelium of frog. Modified Cajal technique counterstained with pyronin-methyl green. In the original preparations the nucleolini are black, the nucleoli (plasmosomes) bright red, and the chromatin green.

Figs. 10 to 16 (with the exception of No. 14) all represent mitotic figures from the columnar cells of the frog's intestine. With modified Cajal-safranin technique employed, the nucleolini alone were impregnated black, chromosomes being stained red and the cytoplasm pink.

Fig. 10.—Early prophase; spireme beginning to segment; nucleolini apparently about to divide.

Fig. 11.—A later prophase, section somewhat oblique, showing equatorial plate and two pairs of nucleolini. Arrows in this and succeeding figure indicate direction of mitosis.

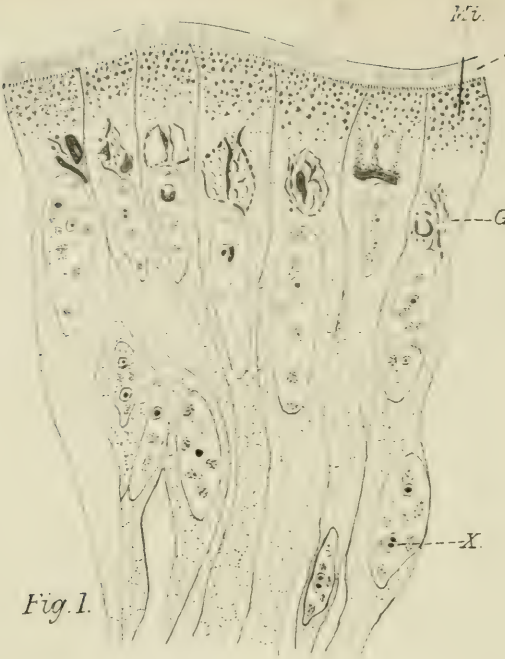
Fig. 12.—Early anaphase, section rather oblique. The two chromosome groups are indicated, each with one pair of nucleolini.

Fig. 13.—Prophase; equatorial plate in transverse section. Only two nucleolini present.

Fig. 14.—Nucleus from intestinal epithelium of frog. Fixed in Bouin and stained in toluidin blue-eosin. The nucleolinus is plainly visible.

Fig. 15.—Late anaphase. Two groups of daughter-chromosomes each with two nucleolini.

Fig. 16.—A still later (telophase) stage. Nuclear membrane reformed. Two nucleolini in each daughter-nucleus.



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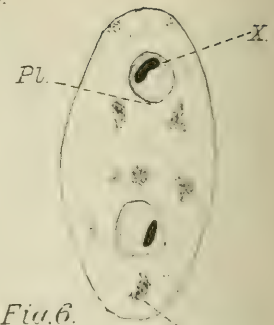
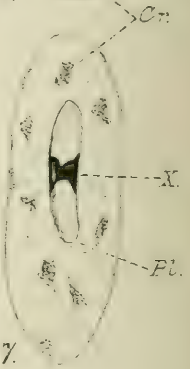


Fig. 6.



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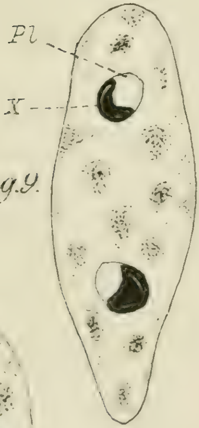

Fig. 9.

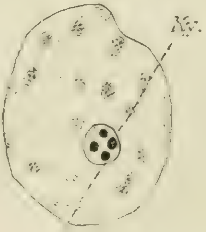

Fig. 4.

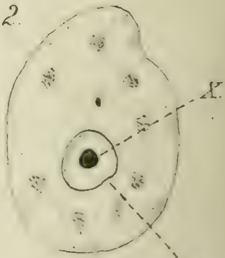

Fig. 2.

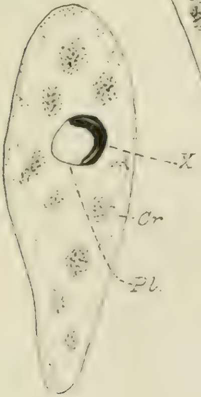

Fig. 8.

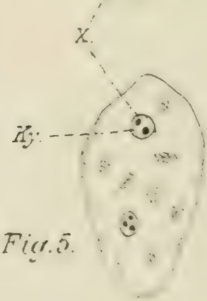

Fig. 5.

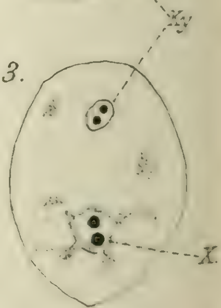

Fig. 3.

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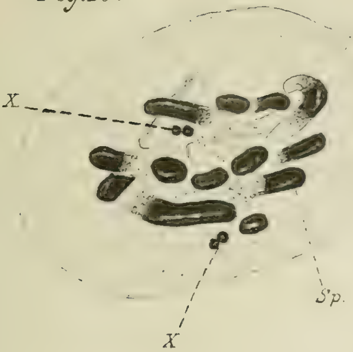
Fig. 10.



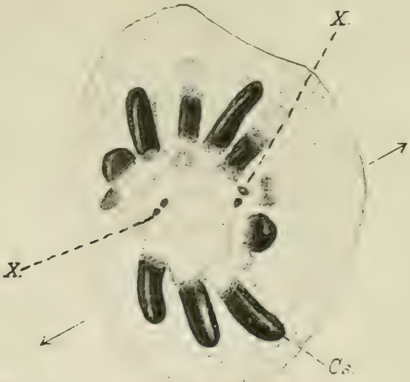
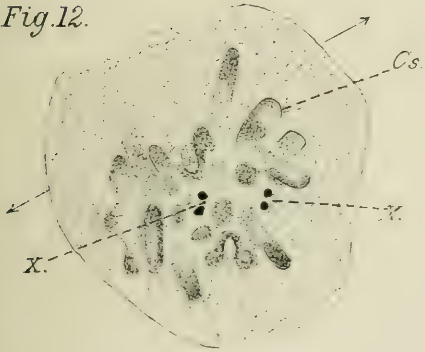


Fig. 11.

Fig. 12.



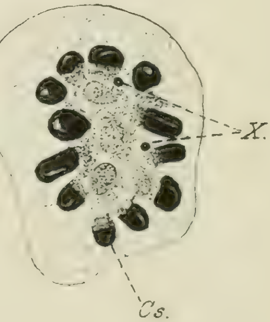


Fig. 13.

Fig. 16.

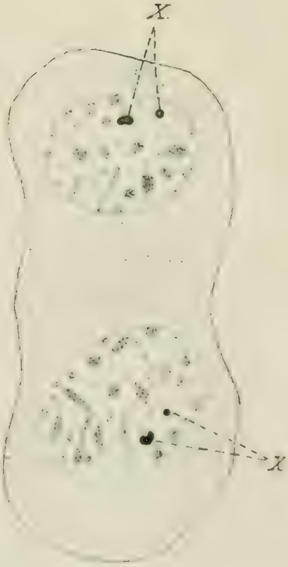
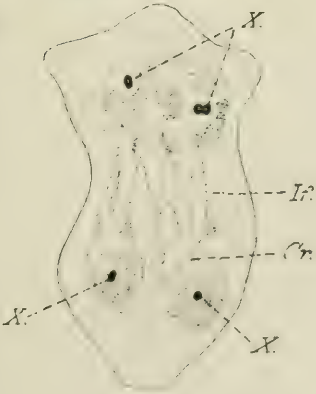


Fig. 15.



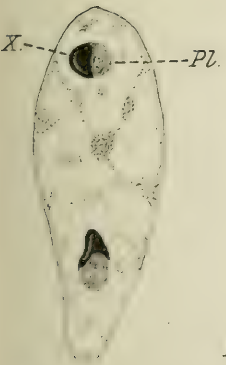


Fig. 14.

Huth. London

CLEOLAR BODY.

The Effects of Ions upon Ciliary Movement.

By
J. Gray, M.A.,
Fellow of King's College, Cambridge.

With 6 Text-figures.

THE experiments described in this paper constitute an attempt to determine the necessary external conditions which support ciliary action. Concerning the mechanism of the cell itself practically nothing is known, and comparatively little work has been done to determine the factors involved by the external medium. In 1902–1906 R. S. Lillie published a series of papers dealing with ciliary action; since then our knowledge of the physical chemistry of solutions of electrolytes has greatly increased, and it seemed desirable to re-open this line of investigation.

Almost from the beginning of the present series of experiments it became apparent that the hydrogen-ion was a factor of supreme importance. Although the work of Sorensen and Palitzch (**6**) has made the determination of the hydrogen-ion concentration of any particular sample of sea-water a comparatively simple matter, yet the conditions which determine this value are by no means clear. Sea-water contains calcium, magnesium, carbonates and phosphates, all of which influence the alkalinity, and at the same time form a complex chemical equilibrium with the carbon dioxide of the air. It will therefore be seen that if the equilibrium is upset by varying the amount of any of these constituents of sea-water the control of the hydrogen-ion concentration of the solution is not an easy problem. It is quite clear that it is not permissible to compare the physiological effects of solutions unless their hydrogen-ion concentrations are adequately controlled—in other words, the qualitative effects of such ions as sodium, calcium or magnesium can only be studied when the hydrogen-ion factor has been excluded. The importance of this factor can hardly be exaggerated.

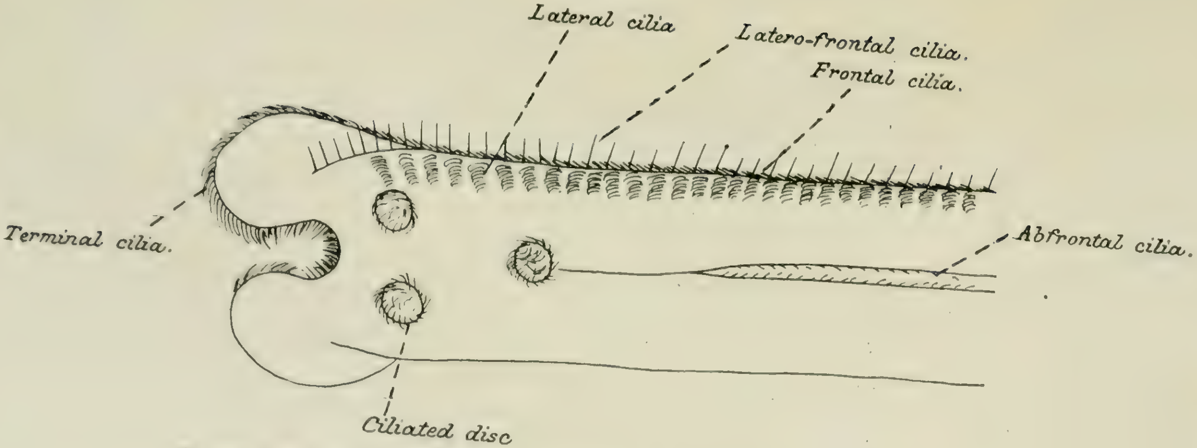
It is admitted that the results obtained neither indicate any specific property of the metallic ions contained in sea-water, nor are they of sufficiently critical value to exclude the existence of such properties. It would appear that such results will only be obtained when it is possible to find satisfactory experimental conditions for controlling the hydrogen-ion concentration of artificial salt-solutions for long periods of time. For example, a solution of isotonic calcium chloride rapidly changes its hydrogen-ion concentration owing to absorption of CO_2 from the air and subsequent precipitation of calcium carbonate. Such a solution, of course, forms an extreme case, but the importance of this point is made evident in the following pages.

The material chosen for this work was the gills of Mytilus edulis. It is simple to obtain, and the gill structure has been carefully observed; further, fragments of gills form a highly satisfactory subject for prolonged experiments. The structure of the gill of Mytilus has been fully described by Orton (**4**), but for the sake of convenience the following summary is given of these observations.

The main inhalent current of water is caused by the lateral cilia. These cilia are exceedingly numerous, and each cell of the epithelium bears several cilia (Text-fig. 4).

It is somewhat difficult to describe the direction of movement of the lateral cilia. Orton states that they "lash across the length of the filaments," but the most characteristic feature of their normal movement is a wave of pulsation which passes along the length of the whole line—the waves pass up on the oral side of the filament and down on the aboral side.

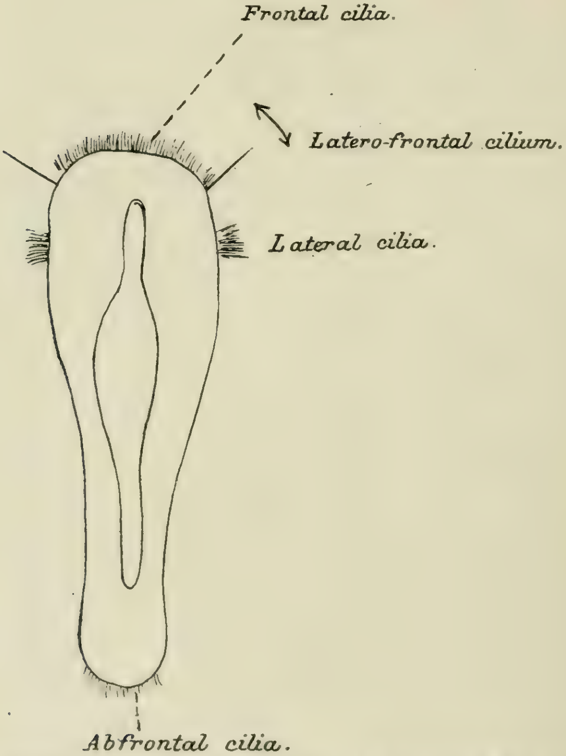
TEXT-FIG. 1.



Lateral view of a living filament of the gill of Mytilus edulis (after Orton).

The latero-frontal cilia are the most conspicuous cilia on the whole gill (ex. the terminal cilia). They stand out stiffly from the filament and move relatively slowly on a line parallel to the transverse axis of the filament. Each cilium arises from a single cell (Text-fig. 5). These are straining cilia and assist in passing food from the main current on to the frontal cilia. The frontal cilia pass the food down the face of the gill on to the ciliated terminal groove. The cilia of the terminal groove lash in an oral-aboral direction and pass the food in a long line of mucus up to the mouth.

TEXT-FIG. 2.

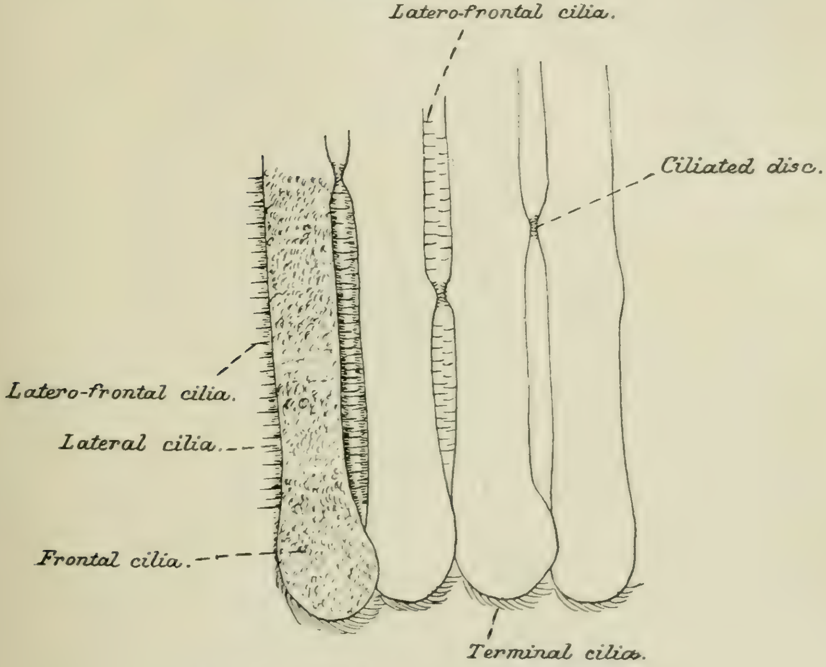


Transverse section of gill-filament of Mytilus (after Orton.)

In studying the effects of various ions on ciliary movement it was therefore decided to select the terminal cilia and the latero-frontals. These cilia have distinct advantages.

(1) The terminal cilia are absolutely free in the solution, and are extremely easy to observe under the microscope.

TEXT-FIG. 3.

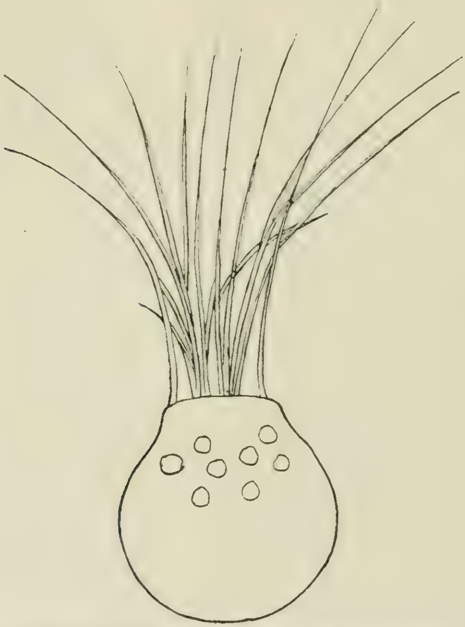


Vertical view of gill-filaments of Mytilus.

(2) The latero-frontal cilia are very obvious, as they are large cilia, and each cilium arises from a single cell.

It is advisable to repeat the recommendation of Orton to the effect that "in the case of all the cilia on gill-filaments, it is necessary to see them living to obtain an accurate idea of their size and function." This is particularly the case with the latero-frontal cilia of Mytilus. In the living gill it is impossible to confuse these cilia with any others—they are much more distinct and move more slowly than any of the others. In preserved specimens they are almost indistinguishable from the lateral cilia. When a portion of the gill is removed the whole of this ciliated mechanism continues—even the co-ordination and transport of the food columns up to the oral corner of the terminal groove.

TEXT-FIG. 4.

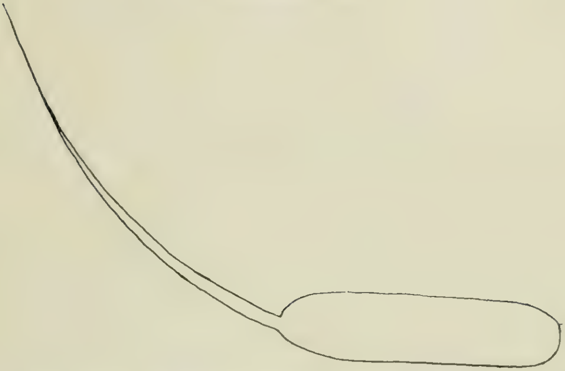


Single cell of lateral ciliated epithelium.

It will be noticed that the ciliary complex of the gill of Mytilus forms a highly co-ordinated system of vital importance to the animal, for on the rate of ciliary movement will depend the amount of food available for nutrition. Such a system one would normally suppose to be closely dependent upon the nervous system of the animal, and that as soon as a portion of the tissue is removed, the mechanism of co-ordinated movement would be speedily deranged. This, however, is not the case. If a single gill-filament be removed and kept in normal sea-water, the whole of the ciliary movement continues unchanged for a very long period. If a portion of the gill-plate $\frac{1}{8}$ in. or more in width be removed, the cilia continue to beat normally for at least fourteen days, with the exception of the lateral cilia, which often cease to show their characteristic waves of pulsation, and may even stop. The latero-frontal, frontal and terminal cilia invariably remain normal.

The reorganisation and regeneration of the ciliated tissue which takes place in isolated gill-fragments after about four days will be described in a subsequent publication.

Text-fig. 5.



Single cell of latero-frontal ciliated epithelium.

During the progress of this work two distinct phenomena have arisen: (1) The action of certain ions upon the activity of individual ciliated cells—i.e. upon ciliary movement as such; (2) the action of certain ions upon the tissue complex. Thus the absence of certain ions results in the break-up of the ciliated epithelium into its constituent cells, although the cilia of the individual cells continue to beat strongly when the cells are entirely detached from the gill and from other cells. It is only in the case of the hydrogen-ion that direct action upon ciliary movement can be studied without injury to the general structure of the gill.

In these pages the following abbreviations will be used :

Terminal cilia . . .	Terminals.
Latero-frontal cilia. . .	Latero-frontals.
Lateral cilia	Laterals.
Hydrogen-ion concentration .	P_H.

For the sake of simplicity the hydrogen-ion concentration will be referred to as "high" or "low," according to the numerical value of the exponent P_H—for example, a solution of $P_H = 9·0$ will be regarded as having a higher hydrogen-ion concentration than a solution for which $P_H = 7·0$. This, of course, involves a contradiction in terms, but it greatly simplifies the text for any reader not intimately conversant with these particular problems.

The term "disintegration" will be used to denote the separation of the individual ciliated cells of the gill epithelium which takes place in many solutions.

Acids and Alkalis.

The sea-water used for these experiments was obtained from the English Channel, some miles south of Plymouth; the hydrogen-ion concentration was constant about 7·85 during the whole period of the experiments. The hydrogen-ion concentration of the various solutions used was determined by Sφrensen's and Palitzsch's method, and the salt correction of these authors was applied in each case.

The effect of lowering the P_H of the sea-water on the ciliary movement of the gills is shown in the following table.

The table shows clearly the profound effect of the hydrogen-ion on ciliary activity. In solutions whose P_H is below about 6·0 the cilia very rapidly came to rest. There is no sign of the ciliated epithelium breaking up, and, as will be shown later, the stoppage of the cilia is entirely reversible when the P_H of the water is raised.

In solutions of P_H 6·7 there appears to be a certain amount of regulation to altered conditions. Thus in sea-water of $_H$ 6·7 the rate of ciliary movement is greatly depressed after about twenty minutes, after which the rate gradually

TABLE I.

50 c.c. sea-water + c.c. N/10 HCl.	P_H.	Ciliary movement after—								
		5′.	10′.	20′.	30′.	40′.	60′.	90′.	120′.	16 hrs.
·14 . .	7·34	++	++	++	++	++	++	++	++	++
·28 . .	6·9	++	++	++	++	+⊕	++	++	++	++
·42 . .	6·7	++	++	+⊕	+	⊕	+⊕	+⊕	+⊕	++
·56 . .	6·6	++	+⊕	⊕	⊕	very slight	very slight	⊕	+⊕	++
·70 . .	6·2	++	+⊕	⊕	⊕	very slight	very slight	very slight	slight	++
·98 . .	5·5	+	⊕	⊕	0	0	0	0	0	0
1·19 . .	4·2	very slight	0	0	0	0	0	0	0	0
1·33 . .	3·8	0	0	0	0	0	0	0	0	0
1·40 . .	3·6	0	0	0	0	0	0	0	0	0

++ Very active.
+⊕ Active.
+ Considerable movement.
⊕ Slight.

increases until after sixteen hours the rate of movement is equal to that of the control. In the more acid solutions no recovery takes place, however, and the tissue eventually loses its translucent appearance and dies. With different individuals the critical P_H differs, but in each case the critical value is well marked; for example, in the case of one gill, movement persisted in 50 c.c. sea-water + 1·36 c.c. N/10 HCl for twenty minutes, whereas it stopped instantly in 50 c.c. sea-water + 1·4 c.c. N/10 HCl; in the case of another gill, movement persisted in 50 c.c. sea-water + 0·7 c.c. N/10 HCl, and stopped in 50 c.c. sea-water + 0·84 c.c. N/10 HCl. Such extreme values are rare, and the average critical value for the N/10 HCl is about 1·12 c.c. to 50 c.c. sea-water.

Before dealing further with the effect of acids, it is convenient to consider the effect of alkalis. If strong alkali is added to sea-water only a comparatively small change is effected in the P_H of the solution before magnesium and calcium begin to be precipitated. At this point a coloration is given with thymolphthalein, equivalent to a P_H of about 9·6. In all such solutions ciliary movement continues actively and usually more actively than in the control experiments. The alkali, however, causes marked disintegration of the tissues, and the individual ciliated cells break away with their cilia still beating strongly. Disintegration occurs in 50 c.c. sea-water + 1·68 c.c. N/10 NaOH or in 50 c.c. Van't Hoff solution + 0·30 c.c. N/10 NaOH.

It is therefore impossible to determine the duration of ciliary movement in hyperalkaline solutions, owing to the fact that the movement continues longer than the tissue retains its normal condition. Even when disintegration is far advanced individual cells continue to move actively. Such isolated cells are in an entirely different environment to that afforded by their normal position in the tissues, and therefore the duration of movement of their cilia can hardly be compared to that of cells remaining in their normal positions. It is interesting to note that individual cells rapidly become spherical in shape; eventually the cilia disappear and the cell cytolyses.

The cessation of movement caused by acid is entirely reversible by alkali if the acid treatment has not been too severe.

Portions of a gill were placed in 50 c.c. V. Hoff's solution + 0·32 c.c. N/10 H_2SO_4 for two minutes; examination under the microscope showed that all movement had stopped. They were then transferred to the following solutions and examined after twenty minutes:

TABLE II.

50 c.c. Van't Hoff's solution. +	Behaviour of cilia after 20 minutes.
0	Not moving.
0·05 c.c. N/10 H_2SO_4	,, ,,
0·10 ,, ,, .	,, ,,
0·05 ,, N/10 NaOH	Active normal movement.
0·10 ,, ,, .	,, ,, ,,
0·20 ,, ,, .	,, ,, ,,
0.40 ,, ,, .	,, ,, ,,

This experiment was repeated many times with the same results except that in certain cases recovery took place in normal Van't Hoff's solution, while in others recovery did not take place so completely in the weaker concentrations of alkali as it did in the stronger solutions. One point was noticeable, however, namely, that when recovery took place in any solution eventually the rate of ciliary movement nearly always became absolutely normal.

It was found that in the case of a certain gill ciliary movement stopped in less than two minutes in 50 c.c. sea-water + 1·26 c.c. N/10 HCl, and that no recovery took place in this solution. Portions of this gill were left in the acid solution for various periods of time and were then transferred to normal sea-water and the time required for recovery noted.

TABLE III.

Time of exposure to acid solution.	Time required for full recovery in normal sea-water.
2 mins.	40 mins.
5 ,,	55 ,,
10 ,,	55 ,,
15 ,,	50 ,,
20 ,,	40 ,,
30 ,,	30 ,,
40 ,,	30 ,,
60 ,,	Disintegration set in, only partial recovery.
90 ,,	,, ,, ,,
120 ,,	Only a few cells recovered.
180 ,,	No recovery.
200 ,,	,,

From the experiments performed it is not safe to draw any definite conclusions except that prolonged exposure prevents ultimate recovery, but it would seem that the length of time required for recovery in sea-water is not dependent upon the length of exposure to the acid solution, and it would seem that the acid produces its maximum reversible effect almost at once, after which a certain amount of regulation takes place within the cell, so that cells exposed to the acid solution of thirty minutes recover just as quickly, if not more so, as cells exposed to the acid for only two minutes.

In their rate of recovery different gills also exhibit differences.

TABLE IV.

	Duration of movement in 50 c.c. sea-water, + 1·26 c.c. N/10 HCl.	Rate of recovery in sea-water after exposure to acid solution for 20 mins.
Gill A . .	< 2 mins.	40 mins.
Gill B . .	7 ,,	20 ,,

In other words, Gill B seems to have been more resistant to the acid solution than Gill A.

EFFECTS OF "NEUTRAL" SALTS.

The fact that the hydrogen-ion has a profound effect upon ciliary movement at once raises a complication when dealing with artificial solutions of the various salts contained in sea-water. For example, a solution of pure sodium chloride in distilled water gives a bright red colour with neutral red, whereas sea-water is usually orange. The reason for this is, of course, to be found in the CO_2 of the air. In the absence of any buffer there is a sufficient concentration of carbonic acid in the sodium chloride solution to give an acid reaction to neutral red or to methyl red. The following table shows the wide variation in hydrogen-ion concentration of the various single salts found in sea-water:

TABLE V.

Salt.	Indicator.	
	Methyl red.	Neutral red.
NaCl	Bright red . . .	Bright red.
,, after boiling .	Orange red . . .	,,
KCl	Red, with orange tinge .	,,
,, after boiling .	Orange	,,
$MgCl_2$	Orange red . . .	,,
,, after boiling .	Orange	,,
$CaCl_2$	Yellow	Red.
,, after boiling .	,, ...	Yellowish-orange.

The salts used were as follows:

NaCl	Kahlbaum (for analysis).
„	Hopkins & Williams (puriss).
KCl	Merck's extra pure.
$CaCl_2$ (anhydrous)	Merck's.
„ („)	Kahlbaum (for analysis).
$MgCl_2$	„ „

It is clear, therefore, that solutions of the above salts possess hydrogen-ion concentrations widely different from each other and from sea-water, and that in order to compare the physiological effects of such pure solutions it is first necessary to adjust these differences in their P_H.

In the case of pure NaCl or KCl the problem presents very little difficulty, as they can readily be buffered by the presence of a very small quantity of sodium or potassium bicarbonate; further, the addition of these salts adds no fresh ions to the solutions. By adding the requisite amount of bicarbonate, solutions can be obtained of the required hydrogen-ion concentration and such solutions can be directly compared with sea-water of the same P_H.

The case of magnesium and calcium chlorides is, however, different. Thus, the addition of sodium bicarbonate to a solution of $MgCl_2$ (such as was used in these experiments) never increases the P_H sufficiently to give an orange colour with neutral red. Again, whereas the addition of strong alkali to a solution of NaCl rapidly gives a bright blue coloration with thymol-phthalein, with $MgCl_2$ only a very faint coloration is produced, and the addition of excess of alkali only causes the precipitation of magnesium hydrate without increasing the hydroxyl-ion concentration of the solution. Similar difficulties arise when dealing with mixtures of NaCl and $MgCl_2$ which never possess a P_H of more than about 7·8 when carbonates are present.

The case of $CaCl_2$ is still more difficult. When the pure anhydrous salt was dissolved in distilled water the solution was found to be very strongly alkaline—in fact, 50 c.c. required 8·25 c.c. N/10 HCl to neutralise to neutral red.

Every sample of anhydrous $CaCl_2$ obtainable gave similar results; this alkalinity is doubtless due to the presence of calcium carbonate.

There is, however, a further complication to be considered in the case of magnesium and calcium chlorides. As is well known, the stabilisation of the hydrogen-ion concentration of a solution containing carbonate depends upon the ratio $\frac{HCO_3'}{CO_3''}$; the higher the value of this ratio the greater the alkalinity of the solution. Now magnesium carbonate, and to a much greater extent calcium carbonate, is much less soluble and less ionised than the respective bicarbonates. Hence the presence of magnesium- or calcium-ions in the solution profoundly affects the number of free CO_3'' ions which can exist in solution, so that these metals also affect the hydrogen-ion concentration of the solutions to which they are added. By increasing or decreasing the number of magnesium- or calcium-ions the hydrogen-ion concentration of the solution is unavoidably altered.

This complication of the problem is by no means simple, and at the present moment it is only possible to bring forward evidence as to the effect of the various metallic ions between fairly wide limits of P_H, and until it is found possible to stabilise the P_H of artificial solutions containing varying amounts of calcium or magnesium, it is impossible to state with exactness the qualitative properties of these ions on physiological processes.

A. ISOTONIC SOLUTIONS OF SINGLE SALTS.

Effects of Solutions of Pure Sodium Chloride.

(1) Pure sodium chloride quickly depresses the rate of ciliary movement, but before cessation of movement is complete disintegration of the tissues begins. The cilia do not appear to dissolve.

(2) Disintegration is well marked in solutions whose P_H is as low as 7·0.

(3) The duration of movement depends partly on the hydrogen-ion concentration of the solution.

TEXT-FIG. 6.

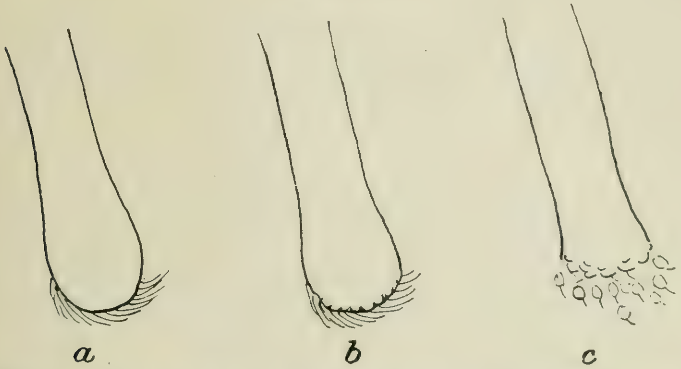


Diagram showing progressive disintegration of the terminal ciliated epithelium in pure sodium chloride.

TABLE VI.

Solution.	P_{H}.	Duration of ciliary movement.	Remarks.
50 c.c. ·6 M. NaCl	7·0	30–120 mins.	In about 10 mins. movement much slowed down; in less than 30 mins. disintegration begins. After 1½–2 hours the latero-frontals and terminals show advanced disintegration, although a few cilia are still moving. After 16 hours the gill is completely disintegrated and all movement of cilia has ceased.
50 c.c. ·6 M. NaCl + (·07–·7 c.c. N/10 $NaHCO_3$)	7·9	30–120 mins.	After 16 hours gill completely disintegrated (a few cells twitching).
	9·0	30–120 mins.	Disintegration well marked after < 30 mins. (a few cells twitching).
·6 M. NaCl + acid (N/10 HCl)	4·0	30 mins.	Movement stops completely. No disintegration.
	3·3	10 ,,	Ditto.
	2·9	4 ,,	,,

(4) In solutions of P_H 7 and higher it is impossible to determine the duration of ciliary movement, as disintegration sets in before the cilia have ceased to move.

(5) The higher the alkalinity of the solution the more intense is the disintegration of the tissues.

(6) The depressant effect of sodium chloride was not removed by subsequent addition of magnesium chloride (cf. Mayer (**3**)).

Effects of Pure Potassium Chloride.

(1) The effects of potassium chloride solution appeared to be almost identical with those of sodium chloride, except that the depressant effect on the rate of movement was less marked.

(2) Disintegration occurs as in sodium chloride, but the individual cells exhibit stronger ciliary movement than in sodium chloride.

Table VII.

Solution.	P_H.	After 2 hours.	5 hours.	16 hours.
·6 MKCl	7·0	Active movement, but progressive disintegration with increasing P_H	Cilia still moving, but disintegration well advanced. Individual cells active	No movement, and complete disintegration in all solutions.
	7·4			
	7·6			
	8·1			

Pure Magnesium Chloride Solution.

As mentioned on p. 357, when sodium bicarbonate is added to a solution of magnesium chloride the P_H never rises sufficiently to give an orange colour with neutral red. Even the addition of strong alkali only produces a faint colour with phenolphthalein. The observations given below must be interpreted in the light of these facts.

Table VIII.

Solution.	P_H.	Remarks.
·6 M. $MgCl_2$.	6·8	Fairly strong movement after 1½ hours. Disintegration began after 2 hours.
„ +alkali	7·8	Strong movement for 1½ hours. A few cilia moving after 16 hours. Advanced disintegration after 16 hours.
„ „	8·0	Ditto.

(1) Normal movement continued for about one hour, after which it slowed down. A few cells continued to move after 16 hours.

(2) Disintegration sets in after about two hours.

(3) In solutions of P_H 6 and less movement rapidly stopped. On transference to Van't Hoff's solution (P_H 8·0) recovery rapidly took place. No recovery took place on addition of NaCl. P_H(7·0).

Solutions of Pure Calcium Chloride.

In calcium chloride solutions movement usually ceases within one hour, but in solutions whose P_H is about 7·8 movement may persist for more than sixteen hours. In solutions of P_H above 6·0 there is a distinct tendency for individual filaments to separate from one another.

Table IX.

Solution.	P_H.	Duration of movement.	Remarks.
·6 M. $CaCl_2$	10·5	< 1 min.	—
	8·3	5 mins.	Gill-filaments separated from each other.
	7·8	> 45 „	Tendency for gill-filaments to separate. Some movement after 16 hours.
	7·6	12 „	Gill-filaments separated from each other.
	6·0	< 1 min.	Recovered in Van't Hoff, P_H 8·0. No recovery in „ „ P_H 6·0.

As mentioned above, the P_H of calcium chloride solutions do not remain constant for more than a few hours owing to reaction with the CO_2 of the air, so that critical experiments are not possible.

B. Binary Mixtures.

(i) Mixtures of Sodium Chloride and Magnesium or Calcium Chloride.

(1) The tissue remains more healthy than in solutions of pure sodium chloride, and ciliary movement remains strong when disintegration is relatively far advanced. Freed individual cells move strongly for some time.

(2) The higher the P_H the more marked is the disintegration.

(3) No apparent difference in effect is noticeable when the relative concentrations $\frac{Na'}{Mg'' \text{ or } Ca''}$ is varied from $\frac{50}{10}-\frac{50}{2}$.

(4) Occasionally cilia remain twitching after forty-eight hours.

TABLE X.

Solution.	P_H.	Remarks.
50 c.c. ·6 M. NaCl	7·3	After 16 hours cilia active; some disintegration of lateral epithelium. Gill otherwise normal.
6 c.c. ·6 M. $MgCl_2$ + Sodium bicarbonate	7·3	After 24 hours some disintegration, but cilia still moving; after 48 hours, no movement.
Ditto	7·5	After 16 hours, very active movement. ,, 24 ,, disruption far advanced. ,, 48 ,, complete disruption.
Ditto	7·6	,, 24 ,, ,, ,,

(ii) Mixture of Potassium Chloride and Magnesium or Calcium Chloride.

These solutions resemble binary sodium mixtures in all respects.

Mixtures of KCl and $MgCl_2$.

TABLE XI.

Solution.	P_H.	Remarks.
50·6 M. KCl .	8·0	2 hours, active movement; some disruption.
4 ·6 $MgCl_2$.	,,	24 hours, complete disruption.
Ditto . .	7·6	2 hours, active movement; some disruption.
		24 hours, complete disruption.
Ditto . .	8·6	2 hours, considerable disruption.
	(by addition of KOH)	24 hours, complete disruption; a little movement.

Mixtures of KCl and $CaCl_2$.

TABLE XII.

Solution.	P_H.	After 2 hours.	After 16 hours.
50 ·6 M. KCl + 2 ·6 M. $CaCl_2$	7·6	Normal	Some movement; some disruption; no separation of filaments.
+ 4 $CaCl_2$.	7·3	,,	Fairly active movement; some disruption; no separation of filaments.
+ 6 $CaCl_2$.	7·0	,,	Ditto.
+ 8 $CaCl_2$.	6·8	,,	Ditto.
+ 10 $CaCl_2$.	6·5	Very active; some tendency of filaments to separate	Very little movement; tendency for filaments to separate.
50 KCl + 2 $CaCl_2$[1] .	10·4	Filaments rapidly separated, but cilia moved strongly for some time. Complete disruption and no movement after 24 hours	—

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[1] In this experiment the original $CaCl_2$ solution was used, in the others the solution was previously neutralised to neutral red.

C. TERTIARY MIXTURES.

(i) NaCl, KCl, $MgCl_2$, or $CaCl_2$.

These solutions support ciliary movement for as much as seventy-two hours.

TABLE XIII.

Solution.	P_H.	Movement after—				Remarks.
		12 hours.	24 hours.	48 hours.	72 hours.	
50 c.c. ·6 M. NaCl . 4 c.c. ·6 M. $MgCl_2$. ·9 c.c. ·6 M. KCl .	5·9	Normal	Slow movement	Slight movement	Slight movement	No disintegration.
Ditto + 5 c.c. N/10 $NaHCO_3$	7·3	,,	Medium movement	Medium movement	Medium movement	Progressive disintegration after 24 hours with increasing P_H.
Ditto + 1 c.c. ,,	7·7	,,	Normal	Normal	Normal	
Ditto + 3 c.c. ,,	8·0	,,	Normal, some disintegration	Total disintegration	—	—

(ii) Mixtures of NaCl or KCl with $CaCl_2$ and $MgCl_2$.

These are less efficient than other tertiary mixtures. Movement ceases in less than twenty-four hours, and disintegration is more marked.

D. MIXTURES CONTAINING KCL, NACL, $MgCl_2$, $CaCl_2$, WITH SODIUM BICARBONATE.

A solution containing these salts in the proportions in which they exist in sea-water, and which contains the requisite amount of N/10 $NaHCO_3$ to adjust the hydrogen-ion concentration to about 7·8, maintains gill-fragments in a healthy normal condition for more than five days, after which the experiments were discontinued.

The optimum composition of this Van't Hoff's solution was found to be:

TABLE XIV.

Salt.	Molecular concentration.	Volume in c.c.
$NaCl$	·6	500
KCl	·6	9
$CaCl_2$	·6	9
$MgCl_2$	·6	36
$NaHCO_3$	N/10	5

SUMMARY OF EFFECTS OF METALLIC IONS IN SEA-WATER.

As far as the action of metallic ions is concerned, the most definite point established is that an efficient physiological solution must contain sodium, potassium, magnesium and calcium. In such a solution, whose P_H is identical with sea-water, gill-filaments will remain healthy and exhibit active ciliary movement for more than five days. If any one ion be omitted from the solution, the ciliated epithelium exhibits signs of disentegration after some hours, and the tissue gradually breaks up, although some cilia may continue to beat for as long as three days. If two ions are omitted, the duration of active ciliary movement is from sixteen to twenty-four hours, but the phenomenon of disintegration is very marked. Finally, in solutions containing only one metallic ion, disintegration rapidly sets in and the tissue is very unhealthy after twelve hours; also ciliary movement is usually markedly affected in less than two hours.

It will be noted that very little evidence is obtained on the qualitative value of any particular metallic ion. Sodium appears to be more toxic than potassium. The presence of excess of calcium appears to cause separation of the gill-filaments, and it is possible, therefore, that this ion affects the ciliated junctions or discs. No clear evidence is obtained of antagonistic action between ions; the action of the various metals appears to be additive. Solutions of pure salts are more toxic than binary mixtures, binary mixtures more toxic than tertiary mixtures, while solutions containing Na, K, Mg and Ca form satisfactory physiological solutions.

A. G. Mayer (3) has described the effect of various ions upon ciliary movement, using annelid larvæ and veligers. He came to the conclusion that ions act upon ciliary movement in exactly the reverse way to their action on neuromuscular activity. His results may be summarised as follows:

Table XV.

Ion.	Nature of action on ciliary movement.
Na' . .	Most powerful of all ions as inhibitor of ciliary movement.
Mg'' . .	,, ,, stimulant ,, ,,
K' . .	Primary depressant, but recovery afterwards takes place.
Ca'' . .	Weak stimulant in presence of Na'.
Weak acids	Primary depressant, but recovery takes place.
NH'_4 . .	Ciliary movement first stops and then recovers.

Mayer points out that these conclusions do not apply to ciliated plant cells.

This author only deals very shortly with the effects of acids, and these were all weak acids—e.g. CO_2, lactic acid, uric acid—and he does not deal with the effect of alkalis except by the statement that NaOH is a primary depressant. He does not mention whether the artificial solutions used were acid or not.

Had the hydrogen-ion concentration of the solutions of the various pure salts been ignored in the experiments described in this paper, it will be noted that the order in which these salts would maintain ciliary movement was as follows:

Magnesium chloride.
Potassium chloride.
Sodium chloride.
Calcium chloride.

It is interesting to note that on this basis one would have agreed with Mayer's conclusions that magnesium is the most efficient ion for maintaining ciliary action, that sodium is a powerful depressant, that pure calcium rapidly stops ciliary movement, and that calcium inhibits the action of sodium. Finally, potassium occupies an intermediate position between magnesium and sodium.

R. S. Lillie performed numerous experiments with isolated gill-filaments of Mytilus edulis. As I became aware of these experiments after the present work had been completed, it is interesting to note that the results of actual experiments with Na′, K′, Ca″ and Mg″ agree very closely. Lillie also mentions the disintegration phenomena in various solutions, which he observed was inhibited by the hydrogen-ion and accelerated by the hydroxyl-ion. He also observed that movement in pure sodium chloride solution was prolonged by the addition of hydrochloric acid. The results of the present experiments tend to support the conclusion that for each salt there is an optimum hydrogen-ion concentration for ciliary movement; at the same time, exception must be taken to the conclusion that this is evidence of an antitoxic effect between the acid and the salt, since the hydrogen-ion concentration of the original salt solution was not investigated. For the same reason, Lillie's experiments with many di- and trivalent salts cannot be regarded as critical, since the hydrogen-ion concentration of these salts differs profoundly, according to their degree of hydrolytic dissociation.

Note on Antagonistic Ion Action.

A very large amount of work has been performed on the physiological action of various ions. In very few cases, however, has any attention been paid to the hydrogen-ion concentration of the solutions used. In view of the fact that solutions of pure salts or mixtures of salts are never of exactly the same P_H as sea-water (or other external medium of the cell), it would appear that many results which have been attributed to the action of metallic ions may possibly be due either wholly or in part to hydrogen or hydroxyl-ions. One or two examples may be quoted. Loeb (2) showed that by exposing fertilised Echinoid eggs to various solutions, on subsequent transference to normal sea-water "twins" were often produced. The following table (from Loeb) shows the percentage of twins produced in various solutions.

TABLE XVI.

Nature of solution.	Length of exposure to solution before transfer to sea-water.		
	2 hours.	2½ hours.	3 hours.
	Per cent.	Per cent.	Per cent.
Na, K	50	90	90
Na, Ca	5	80	90
Na, Mg	40	80	90
Na, K, Ca . . .	0	½	5
Na, K, Mg . . .	50	90	100
Na, Ca, Mg. . .	1	20	20
Na, K, Ca, Mg. . .	0	0	0

Further, Loeb showed that the effect of the simpler solutions was inhibited by alkali.

TABLE XVII.

Solution.	Per cent. of twins produced.
Na, K	50
50 Na, K, ·1 M/5 $NaHCO_3$ . .	0
50 Na, 5 Mg.	98
,, + ·1 M/5 $NaHCO_3$	0
50 Na, 5 Ca	15
,, ,, + ·1 $NaHCO_3$ . .	0
50 Na, 5 Mg, 1·1 KCl	60
,, ,, ,, + ·1 $NaHCO_3$	0

Now, when it is remembered that $CaCl_2$[1] may be distinctly alkaline, that $MgCl_2$ is more alkaline than KCl, and that NaCl is distinctly acid, the above results are capable of a very simple explanation. If the production of "twins" is due to exposing the eggs to a solution of low P_H, then in Table XVI we would expect to find that all those solutions which lack calcium produce a high percentage of twins, whereas solutions containing calcium do not. This is actually the case except in the case of eggs exposed to Na and Ca for three hours; but Table XVII shows that the effect of this solution also is removable by raising the alkalinity.

Again, in the case of Osterhout's (5) results on plant growth:

TABLE XVIII.

Solution.	Duration of life.	Solution.	Duration of life.
NaCl	23	$CaCl_2$	58
KCl	56	NaCl, $CaCl_2$	65
$MgCl_2$	19	NaCl, $MgCl_2$, $CaCl_2$	45
NaCl, KCl	23	NaCl, KCl, $CaCl_2$	88
NaCl, $MgCl_2$	25	NaCl, KCl, $CaCl_2$, $MgCl_2$, $MgSO_4$	∞
NaCl, $MgCl_2$, KCl	30		

In these experiments no mention is made of the hydrogen-ion concentration or of the effects of alkalis. In each case the solutions containing calcium support life very much better than those without this metal.

It is not necessary to enlarge upon the critical effects of very small changes in the hydrogen-ion concentration of the medium with which an organism or cell is in contact. The work of Mines, Moore, etc., amply illustrate the point. It is therefore necessary to question the conclusions drawn from experiments dealing with antagonistic ion action in which the hydrogen-ion concentration of the various solutions has not been controlled. It is, however, not suggested that changes in hydrogen-ion concentration afford a complete explanation of such experiments, or that true antagonistic ion action does not exist.

---

[1] While anhydrous calcium chloride tends to be alkaline, the crystallised salt is usually more acid than sodium chloride solutions, and the above explanation would not apply.

Summary of Results.

(1) With the exception of the wave action of the lateral cilia, isolated fragments of the gills of Mytilus continue to function normally in sea-water for many days.

(2) Ciliary activity is dependent upon a certain minimal concentration of hydroxyl-ions ($P_H = 5{\cdot}5 - 6{\cdot}0$).

(3) Stoppage of the cilia by acid is reversible by raising the P_H by means of alkali.

(4) If the P_H of the medium is above 9·0 the ciliated epithelium rapidly breaks up into its constituent cells, but ciliary movement does not stop either in the isolated cells or in those which remain in situ.

(5) The breaking up or disintegration of the ciliated epithelium takes place in all solutions which do not contain potassium, sodium, magnesium and calcium.

(6) Solutions containing only one metallic ion are highly toxic to the tissue, causing marked disintegration even at low values of P_H. Solutions containing two ions are less detrimental than solutions containing only one. Solutions containing three ions support the tissue in a comparatively healthy state for as much as seventy-two hours; but it is only when the four metallic ions are present that the tissue remains normal and healthy as in sea water.

(7) Little evidence was obtained of qualitative effects of single metallic ions or of antagonistic ion action. Sodium chloride forms the most toxic salt in sea-water.

(8) Attention is drawn to the necessity of controlling the hydrogen-ion concentration in all solutions used in the investigation of antagonistic ion action.

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Planktothuria diaphana, gen. et sp. n.

By

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With 4 Text-figures.

The subject of the following notes is a transparent gelatinous organism, which was at first put aside with other plankton collections as some form of medusa. A subsequent examination, however, proved it to be a Holothurian with several features of special interest.

About a dozen in all were procured in a non-closing net used in deep water off the Cape of Good Hope, so that the depth at which they occurred is not known. Most of them were somewhat broken up on account of their delicate organisation, but a few were sufficiently perfect to allow of a fairly detailed examination.

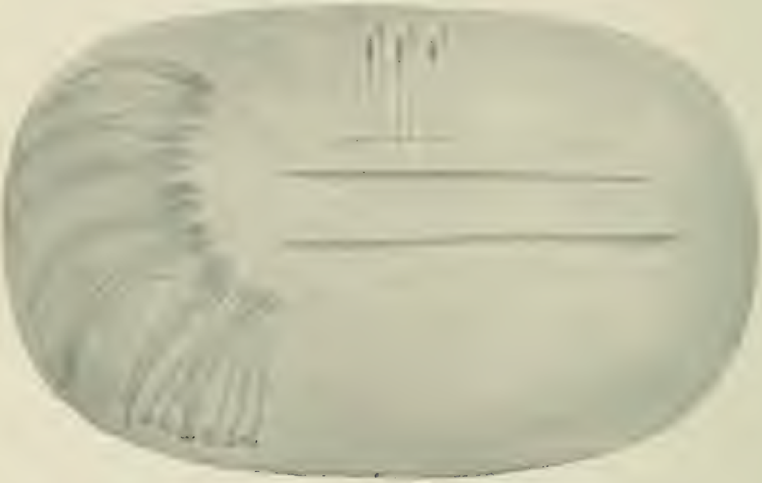
The presence of a well-defined circle of tentacles, circular and radial canals could be readily made out, and were sufficient to indicate Holothurian affinities, the absence of external podia suggesting the family of the Molpadiidæ or Pelagothuriidæ. The fact, however, that the tentacles are supplied from the circular canal is an important feature, and one which renders the classification of the animal difficult.

External Characters.

In the fresh state the whole body is transparent and buoyant. None were alive on being brought to the surface, and they settled slowly to the bottom of the vessel in which they were placed. There was no marked coloration, the body being clear and glassy, like that of a medusa. In the preserved state the body is of a darker colour, and is surrounded by a thick jelly-like mass in which podia and tentacles may be seen embedded (Text-fig. 1).

The largest were about 40 mm. in length, and in one less than this mature ova were found, so that they do not represent young stages. The body is rounded and thick, its breadth being more than half its length. Probably in the living condition it is much more elongate. There is no differentiation of the upper and under surfaces, apart from the position of the mouth and tentacles. The mouth is small and directed more or less ventrally, the anus being terminal, and large and distinct in some individuals.

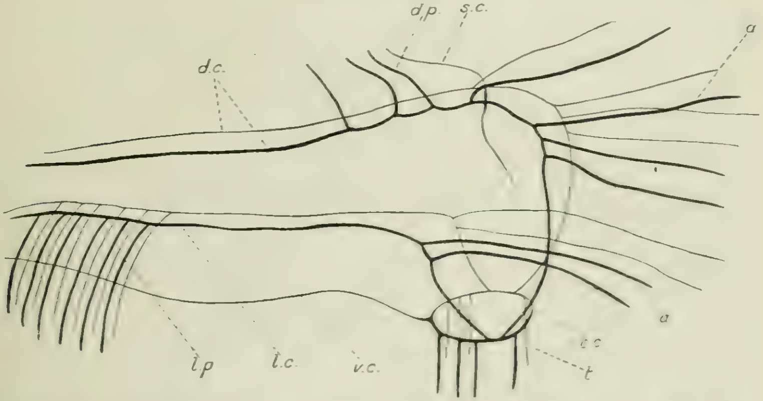
Text-fig. 1.



Planktothuria diaphana (dorso-lateral view), showing arms or anterior podia, tentacles, three pairs of dorsal podia, in front of which are the gonoduct and stone-canal.

Podia.—The podia are all embedded in the thick gelatinous body-wall, and do not project beyond its surface, with perhaps the exceptions noted below. The ventral radial canal has none, but both the two latero-ventrals and the dorsals have them. They are arranged in three distinct groups, which, however, are of mixed origin. Thus at the anterior end of the body they assume the form of a number of arms or tentacle-like structures, arranged in the form of a hood, partly surrounding the mouth-region. On the dorsal region they are delicate and thread-like, while at the posterior end of the body they are more pronounced, and project out laterally from the sides, being approximately at right angles to the body.

TEXT-FIG. 2.



Water-vascular system. *a.*, arms.; *c.c.*, circular canal; *d.c.*, dorsal radial canal; *d.p.*, dorsal podia; *l.c.*, lateral radial canal; *l.p.*, lateral podia; *s.c.*, stone-canal; *t.* tentacles; *v.c.*, ventral radial canal.

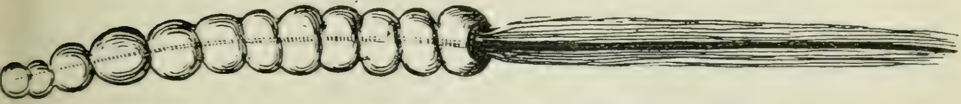
The arms or oral podia are well developed and conspicuous structures, and consist of fine canals of the water-vascular system accompanied by muscular tissue, so that they assume the form of elongate arms, broad and closely appressed at their bases, and tapering off to a fine extremity in a wide curve or arch. They are somewhat flattened against each other near their bases, and, towards their middle, become narrower. The canal lies on the outer side of the muscular tissue, which, in transverse sections, assumes the form of a closed or convoluted layer of tissue with deeply staining nuclei. The origin of the podia from the water-vascular system can readily be made out by a suitable preparation of the whole body. Text-fig. 2 is a camera lucida drawing of a specimen from which the viscera have been removed, and which has been stained sufficiently to show the water-vascular system. It is then seen that these anterior podia (Text-fig. 2, *a.*) arise, partly from the two lateral radials (*l.c.*), and partly from the two dorsal radials (*d.c.*) In this particular individual two arise from each of the lateral radials on each side, and four from each of the dorsal radials.

The dorsal podia (Text-fig 2, *d.p.*) are poorly provided with muscles, and appear only as delicate thread-like structures, apparently incapable of keeping in position unless supported by the surrounding tissue. They are situated in the anterior third of the body, and are usually six in number—three attached to each dorsal radial. .In some small individuals only four were seen, and, in some of the larger, there were indications of additional podia posteriorly. That these dorsal podia, however, have some special functional significance seems to be indicated by the structure of the terminal portions. In the better-preserved specimens they are seen to end in swollen or enlarged structures, usually contracted into an elongate homogeneous mass, but when dissected out and examined microscopically are seen to be of the nature shown in Text-fig. 3. It is seen that the fine tubes and muscular strands of the main part somewhat suddenly become constricted, and then enlarge into a more solid part. This terminal portion is elongate (2·5 mm. in a large individual), and its surface is raised into a number of ring-like folds. Though in all cases this terminal part did not project beyond the surface of the body, it may be that in the living and fully-expanded condition it does so, and perhaps serves some sensory function, for it can hardly be an organ of respiration in view of its small size. Longitudinal sections show a well-defined epithelium, a central canal and muscular tissue, which occurs, however, on the posterior side only.

The lateral podia (Text-figs. 1, 2, *l.p.*) vary in number but are usually six to eight. They are confined to the posterior half of the latero-ventral radials. In structure and appearance they resemble those of the anterior end of the body. Proximally they are broad and flattened in a plane at right angles to the main axis of the body, but at their distal end they taper into a fine point which does not project beyond the surface of the body.

Tentacles.—These are well-developed structures, and their enlarged extremities form a conspicuous feature of the body, each of these being divided into two main branches, from which lateral branches arise (Text-fig. 4, *t.*). The tentacles vary in number and apparently increase in size and number with the age of the animal, 8 being found in the smallest and 16 in the largest.

Text-fig. 3.



One of the dorsal podia, showing the modified terminal portion.

The origin of these tentacles from the water-vascular system can readily be made out, both in the entire animal and in stained preparations. It was seen that they arose entirely from the circular canal. To make this important fact quite certain the ring-canal was dissected out, stained, and mounted whole (Text-fig. 4), and in another case was sectioned, and the direct connection between tentacles and canal confirmed. The point of origin of the tentacle from the circular canal has no definite or constant relation to that of the longitudinal canals. In the case figured two arise between the dorsal radials, three and six on each side of the ventral radial.

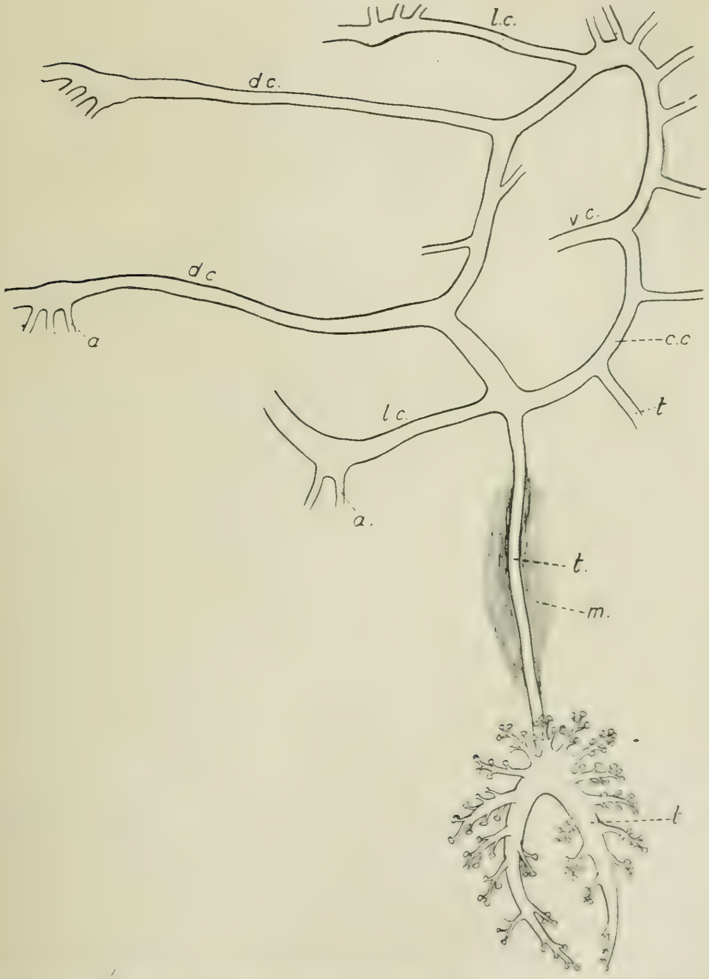
Stone-canal.—Slightly in front of the dorsal podia, and in the mid-dorsal region between the two radials, may be seen two slender tubes, not very different in appearance from the dorsal podia (Text-fig. 1). The posterior of these leads to the gonads, and that in front (Text-fig. 2, *s.c.*) to the circular canal, and is apparently the stone-canal. It opens by a single opening on the surface of the body, and is continued inwards as a fine tube till it passes the muscular layers, when it expands into a much thicker portion. No calcareous deposits were observed in any part of the stone-canal, the thicker portion appearing in sections as a simple tube with deeply staining walls, more developed on one side than on the other. The stone-canal and its swollen part are indicated in Text-fig. 2. The connection with the circular canal, though seen in dissections of large specimens, was not made out in this preparation, in which it has been broken off, and is, therefore, not indicated in the drawing.

Body-wall.—The body-wall consists mainly of a thick, gelatinous tissue, composed of elongate cells with deeply staining nuclei. It is similar in its nature to that found in some other deep-sea Holothurians, but is more developed, and of a looser and more transparent nature. Below it are the circular and longitudinal muscles of the body. The circular muscles are not well developed, and are interrupted by the longitudinal. The latter are double and are well developed. Retractor muscles were not observed.

Skeletal System.—The skeletal system is poorly developed. No calcareous deposits were found in the body-wall, either in stained or unstained preparations, but round the œsophagus were numerous minute-knobbed spheres, which were about ·0176 mm. in diameter, this size being very constant. Effervescence occurred on the application of an acid, so that doubtless they represent all that remains of a calcareous ring.

Alimentary Tract.—The mouth is situated in the middle of a large peristome which faces somewhat ventrally; in some cases its plane is quite parallel to the long axis of the body. The mouth, therefore, is distinctly ventral in position. The alimentary tract is more or less injured, apparently by

TEXT-FIG. 4.



Anterior region of water-vascular system, showing circular canal (*c.c.*), from which are given off the two dorsal radial canals (*d.c.*), two lateral radial canals (*l.c.*), one ventral radial canal (*v.c.*), and eleven tentacles (*t.*), one of which is drawn in the fully-expanded condition; *a.*, arms; *m.*, muscle accompanying canal of tentacle.

the strong contraction of the longitudinal muscles; in one it has been forced out at the mouth, in another at the anus, and in others the body-wall has been ruptured. In one, however, it was observed that the median dorsal mesentery was attached to the intestine for about two-thirds of the length of the body. The course of the intestine is seen in some to be in the form of a double loop. The wall of the intestine is very thin when distended with food, and when not so distended shows in transverse sections numerous convolutions. There is no trace of a respiratory tree nor a Cuvierian organ, but the rectal portion of the intestine, in one or two cases, is distinctly enlarged and connected to the body-wall by muscular fibres. No diverticulum of this part was observed, but the walls were spongy and soft.

The food found in the alimentary tract consisted of greenish flocculent material, radiolarians, foraminifera, many diatoms and sponge spicules.

Gonads.—In a specimen 30 mm. from mouth to anus the ovaries, judging from the state of the ova, are well developed. They occur at the anterior part of the body, and are attached to the dorsal mesentery. They are in the form of a bilobed grape-like mass, in which the larger ova could be readily seen with the naked eye. The rounded mass is 4 mm. in diameter, and is divided into a right and left half, placed to the right and left of the dorsal mesentery. A short duct passes to the body-wall, which it penetrates, and is continued as a long, thin-walled, very transparent tube, leading through the gelatinous epidermis to the exterior. It passes through the body-wall immediately behind the stone-canal, and not far from the base of the nearest arm (Text-fig. 1).

In a smaller female, 26 mm. in length from mouth to anus, the ovaries were apparently mature, some of the larger ova being found detached in the body-cavity. These mature ova are well characterised. They measure ·3 mm. in diameter, and are surrounded by a clear glassy envelope. In the egg itself occurs a large spherical globule, about ·16 mm. in diameter, resembling a large oil-globule. Besides this large globule several other and much smaller oil-globules occur in groups.

HABITS.

The habits of the animal can only be a matter of conjecture. It is certainly not so obviously adapted to a pelagic mode of life as Pelagothuria, the only Holothurian hitherto known to be free swimming. It has been observed, however, as I have already noted elsewhere ('Marine Biological Report No. iii, Union of South Africa,' 1916, p. 46), that some of the Holothurians procured in deep water off the South African coasts have the power of swimming about freely in the water by an undulatory movement of the body, so that there is nothing new or improbable in the supposition that the form under consideration, which seems to be more adapted structurally for this mode of life, should be pelagic. Probably such deep-sea Holothurians do not bury themselves in the soft mud of the floor of the ocean, but flit more or less readily over its surface.

SYSTEMATIC POSITION.

The chief difficulty in placing these animals in any of the great groups of the Holothuroidea is the fact that the tentacles arise from the circular canal. This character, following Ludwig's classification, would remove them from most of the families of the Holothuroidea (Actinopoda) and bring them into the family of the Synaptidæ (Paractinopoda), from which, however, they must be separated, as this family has neither radial canals nor podia. It would seem also to indicate the necessity of a basis of classification of families of the Holothuroidea other than that proposed by Ludwig.

Apart from this character the animal seems to be nearest to the Elpidiidæ, and may be placed provisionally in this group as a new genus, or otherwise in a new family, the Planktothuriidæ.

SUMMARY.

1. A medusa-like Holothurian has been found off the South African coasts.

2. It is devoid of external podia.

3. Podia are found embedded in the thick gelatinous epidermis, and are arranged in three groups in the form of—

    (1) Arms or oral podia forming a hood-like structure over the mouth, and supplied from the dorsal and ventro-lateral radial canals.

    (2) Dorsal podia, which are slender and terminate in knobs.

    (3) Lateral podia towards the posterior end of the body.

4. Tentacles are supplied by the circular canal.

5. The stone-canal is single, and opens to the exterior by a single opening.

6. Calcareous deposits do not appear to occur in any part of the body, except in the form of small knobbed spheres round the œsophagus.

7. Respiratory tree and Cuvierian organ absent.

8. Gonads in two grape-like masses, one on each side of the dorsal mesentery, and opening to the exterior by a long, slender duct. Mature ova ·3 mm. in diameter, and provided with oil-globules.

9. It cannot be placed in any of the known groups of Holothurians, chiefly on account of the origin of the tentacles from the circular canal.

Protozoa parasitic in Termites.

Part II.—Jœnopsis polytricha, n. gen. n. sp., with Brief Notes on two New Species, Jœnopsis cephalotricha and Microjœnia axostylis.

By

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With Plates 18—21.

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INTRODUCTION.

In an earlier paper (**1**) I described the morphology and life-history of the commonest protozoa, Ditrichomonas termitis, resident in the hind gut of an Indian termite, Archotermopsis wroughtoni, Desn. In the present paper I propose to consider in a similar manner other of these parasites, which have already been partially described by Imms (**10**) under the designation Species β. For reasons given on page 399 I propose to found the new genus Jœnopsis, containing the two species J. cephalotricha and J. polytricha.

A third species has not been described by Imms, and on account of its similarity to Microjœnia hexamitoides (Grassi) I have named it Microjœnia axostylis.

These three species are not so common in the termite as D. termitis, but they appear to occur with equal frequency in the two sexes of all casts. J. polytricha and J. cephalotricha are, however, more prevalent than M. axostylis, which is a relatively rare animal.

METHODS.

The methods adopted in the study of the parasites were broadly the same as those described in my previous paper. The two most useful stains were Heidenhain's iron hæmatoxylin and Dobell's hæmatein described by him in 1914 (**2**). As regards fixatives, Schaudinn's fluid containing 4·5 per cent. acetic acid as recommended by Dobell and Jepps (**3**) and Bouin's fluid are excellent for general use. It was found, however, that both these fixatives affected the parabasal apparatus of J. polytricha, as was recorded in the case of D. termitis. In order to obtain good images of these bodies I employed Fleming's strong fixative from which acetic acid had been omitted as recommended by Gatenby (**5**). For an elaboration of this point reference should be made to my previous paper.

All attempts to obtain artificial cultures of the parasites were unsuccessful.

JŒNOPSIS POLYTRICHA: LIVING CONDITION.

In shape this animal is broadly pyriform with a much rounded anterior end. Measurements of a large number of individuals show that the length varies from 129–95 μ and the width from 47–77 μ. These values are slightly lower than those obtained by Imms, who gives 159–114 μ as the length range and 95–41 μ as that for the width.

About one-third of the distance from the anterior extremity there is developed a well-marked depression or groove, somewhat oblique in position and extending round the whole body, so as to separate the animal into distinct anterior and posterior portions or "head and body" regions (Pl. 18, figs. 1, 2, *G.R.*).

For a long time I thought it possible that this groove might represent a pharynx, but this view is untenable. If the animal is observed in a solution containing powdered carmine the particles which pass into the groove on one side of the body are always ejected in a very short time from the other side, and in many cases the granules can be seen travelling along the floor of the groove in an irregular manner. In no case have I found any evidence that the carmine was ingested or that the depression represented anything more than a furrow in the body-plasma. Further, if a pharynx were represented by the groove one would expect to find food-particles situated in or near to it: this has never been seen though hundreds of specimens have been examined; in all cases the food-particles—wood—are situated towards the posterior end of the body.

At the anterior end of the body there is a solid structure, probably supporting in function which I have termed the supporting structure or piece (Pl. 18, figs. 1, 2, *S.P.*). From it arise two long rods extending through about two-thirds of the body length. They are situated in the middle of the endoplasm and take a somewhat curved course (Pl. 18, fig. 2, *P.T.*).

When I thought that the groove mentioned above represented a pharynx I was inclined to regard these rods as pharyngeal supports, but research has demonstrated that they are totally different in nature and must be homologised with the bodies found in Jœnia annectans and termed by Grassi "il regolo" and "il collare." I prefer, however, to adopt the terminology of Janicki (**11**) and name them "parabasal structures." A more detailed account of the bodies will be found on page 401.

Running over the anterior two-thirds of the body are longitudinal striations, which form a right- and left-handed series (Pl. 18, fig. 2, *S.T.*). Each member of the series takes its origin in the supporting piece of the head, and pursuing a straight course passes over the groove; it then turns either to the right or left side for its remaining length. On account of this when the animal is viewed from the front or back (dorsal or ventral) surface two series of striations are seen diverging from each other in the middle line (Pl. 18, fig. 2), but when seen from the side the whole body-surface appears to be striated (Pl. 18, fig. 1).

At first sight the whole of the body seems to be covered with long flagella; more detailed observation shows, however, that the flagella are arranged in two definite series, which may be termed the "head" flagella and the "body" flagella series (Pl. 18, fig. 2, *H.F.*, *B.F.*).

The first of these series possesses a large number of flagella, arranged in a tuft recalling that found in Jœnia annectans. These flagella are about two-thirds as long as the body and when functioning act together in unison in a whip-like manner, thus serving as the main propulsive organ of the body. Their origin is in the supporting piece of the head (Pl. 18, figs. 1, 2).

The body series of flagella arise from granules situated on the striations, a more detailed account of which I will reserve for a later section of this paper (p. 389). In length the flagella are about equal to those of the head but extend in all directions over the body-surface, taking a backward course.

They are by no means as active as the head-flagella, moving but little when the animal is swimming but maintaining a slow undulatory action when it is at rest. It appears as though their function is similar to that of the undulating membrane of D. termitis described in my previous paper.

Owing to the length of the flagella and to the circumstance that they are placed near to the groove, it often appears as though some of them were actually inserted in it and were analogous to the peristomial or undulating cilia of the Ciliates. This is not the case, for detailed observation shows that there are no flagella actually in the groove and that the erroneous impression is due to the free ends wafting over it.

A well-developed axostyle is clearly visible in the living animal, arising from the supporting piece and passing between the parabasal structures to reach the posterior end of the body, from which it issues to terminate in a sharp point (Pls. 18, 19, figs. 1, 2, 3, 11, *A.X.*). Usually only a small portion of the structure is free from the body but occasionally a relatively large part is seen. I have never observed any retraction or lateral movement such as was described in the living, D. termitis.

MOVEMENT.

The animal is an exceedingly powerful swimmer as might be surmised from its flagellar covering. As I have mentioned above the main propulsive organ appears to be the head series of the flagella, acting in a whip-like way. The whole apparatus first bends down on one side of the body and then rapidly moves upward to the middle line, repeating the process almost immediately. In this way the body is drawn forwards and at the same time rolled over to that side opposite to the one to which the flagella curve themselves during action. Thus while swimming the animal is continually rotating on its longitudinal axis.

Owing to the parabasal structures being slightly curved it is possible to distinguish arbitrarily a right and left side, and for convenience I have called the side towards which the convexity of the parabasals points the left one. It is interesting to note that the flagella usually bend towards this, the left side, and the rotatory motion of the animal is therefore from left to right—that is, clock-wise.

The remainder of the flagella scattered over the body have an entirely different action; they may be seen projecting in all directions from the body edge and are in continuous movement, which is, however, so erratic and feeble that it may be described as quivering. It is difficult to believe that they play any important part in the translation of the animal.

Feeding.

The main food supply of J. polytricha is the food-particles on which the host has been living. In nearly every specimen examined some of the particles have been found in the endoplasm scattered irregularly in the posterior region to which they are confined.

No cytostome is present and I have been unable to detect the method by which the animal obtains its food. As is well known, this is an unsolved problem in the majority of the termite parasites. It has been suggested that the posterior region of the body is capable of producing pseudopodia which engulf the food (Trichonympha), but in Jœnopsis I have never been able to find any evidence that this method obtains, although much time has been spent watching these animals in a fluid to which powdered carmine has been added.

In other experiments the termites were fed with carmine and then after two days killed and their rectal contents examined. Although many of the protozoa contained carmine granules, sometimes in numbers, in no case was it possible to detect the method by which ingestion had occurred.

In this connection a paper by Buscalioni and Comes (**1a**), which appears to have been overlooked by many observers, is of interest. With this work I hope to deal in a future paper, where I shall discuss the possible relationship of the Protozoa to their termite host.

MORPHOLOGY.

(A) Striations and Flagella.

In stained preparations there can be seen no differentiation into ectoplasm and endoplasm, but there is a delicate membrane to the body sufficient in strength to prevent variation in the shape of the animal. The cytoplasm is in most cases granular, vacuolated protoplasm rarely being encountered. As already mentioned, the wood-particles are confined to the posterior region of the body and are not surrounded by a distinct food-vacuole.

In preparations stained by Heidenhain's method the striations are very clearly seen. Their general arrangement has been already discussed, but when stained a number of interesting points are revealed.

In the first place it is noticed that the anterior two-thirds of the body is raised into a series of ridges, having their origin in the supporting piece of the head, in a manner similar to that described by Hartmann (**9**) in the Form A of his Trichonympha, now referred by Grassi (**7**, **8**) to the genus Pseudotrichonympha.

Each ridge of J. polytricha continues for a short distance as a narrow line, which broadens considerably, until at its thickest portion it measures about 3·9 μ. Towards its posterior extremity narrowing again takes place so that ultimately a short thick thread is seen similar to the anterior one (Pl. 18, fig. 3, *S.T.*). The whole complex reaches a length varying in different animals from 67·7–76·8 μ.

The striations seen in the living animal are the sides of these ridges, which, narrowing at the anterior and posterior extremities, give the appearance of a single line. Thus in the middle of the body each pair of striations corresponds to one ridge. Reference to Pl. 18, fig. 4, which is an enlarged drawing of two ridges, will render clear the above account.

On the broad part of the ridges are situated numerous deeply-staining granules from each of which one body-flagellum takes origin (Pls. 18, 19, figs. 4, 11, *B.G.*).

Transverse sections through the body show that these flagella are arranged on crests of the body-surface (Pl. 19, fig. 8), and not in depressions, as has been described so often in the Ciliates, where striations are so commonly found. Probably a delicate membrane covers the granules, though this was not observed in the sections.

It is possible that the activity of the flagella in Jœnopsis may have caused this raising of the portions of the body-surface, through the continuous tension which they exert. It is true that their movement is feeble but they are so numerous and so closely placed together that their combined effect must exert considerable force on the delicate surface of the body.

In view of this suggestion it is of interest that at those places where the flagella are most numerous, and where the tension is therefore greatest, there the ridges are broadest, while at the anterior and posterior narrow portions the flagella are few.

In the same way the large tuft of head flagella may have produced the broad papilla, which I have termed the head, by their continual pulling on the protoplasm.

These head flagella arise from numerous basal granules arranged in two series, one on each side of the supporting piece (Pls. 18, 19, figs. 5, 11). This structure stains deeply with iron-hæmatoxylin, but readily gives up its colour when suitably treated, leaving the basal granules deeply stained. This affinity for stain was also found by Grassi (**8**) to obtain in the complicated supports—“complicati sostegni”—of Staurojœnina mirabilis.

(B) Parabasal Bodies.

Also from the supporting piece there arise the two bodies which I have already mentioned as homologous to “il regolo and il collare” of Jœnia, and which I have termed the “parabasal threads.” In stained preparations they colour deeply and are not easily decolorised by the ordinary reagents used for this purpose. The length varies from 66·6–80·8 μ according to the length of the entire animal and they are slightly curved. Their shape is rectangular, with the long side of the rectangle much greater than the short one. Measurements of these bodies in transverse section gave the relation of the two sides as above 3·1. Towards their anterior end, however, the long side narrows, becoming almost entirely equal to the short one, thus giving the appearance of a thick thread about 1·6 μ in diameter (Pl. 18, figs. 3, 5, *P.T.*). The two bodies are not arranged in the same plane as that of the supporting piece but transverse to it, so that one of the bodies is always at a lower level than the other—usually 7 or 8 μ deeper in the body. Also they run through the middle of the plasma in no way superficially, so that in order to obtain a sharp focus of the deeper body it is necessary to lower the objective of the microscope 20–30 μ below the level required for body surface focus.

In making preparations the pressure of the cover-slip and glass causes flattening of the animal with the result that the parabasal threads come to lie practically in the same plane; during this process the lower one is rotated on its axis giving an appearance such as is shown in the figures, where the narrow side of the upper parabasal is seen, but the long side of the lower one.

Situated on the broad surface of these parabasal threads there are numerous rod-like structures arranged irregularly; their length being about 6·8 μ and their diameter about 1·2 μ. They are most numerous on the median and posterior extremities, but a few are found up to the point of origin of the supporting piece. On account of their situation their whole length is seen projecting outwards in all directions only on that thread whose short side is viewed—that is, the upper one, while in the lower one the rods are seen as small rounded structures (Pls. 18, 19, figs. 5, 9).

I think there is no doubt but that these rods represent "le figlioline" described in Jœnia by Grassi (6, 7).

A short discussion of the probable homologies of these bodies will be found on p. 401.

Finally I may mention that these rods are only distinctly seen in those preparations fixed with Fleming's fluid as modified by Gatenby, or by a fixative containing neither corrosive sublimate or acetic acid. In preparations fixed by fluids containing these substances the rods are very variable as regards their presence, and at all times they are very indistinct in outline. An exactly similar effect was found in D. termitis, as described in my previous paper, where the whole question is briefly discussed.

(c) Nucleus.

Between and at the base of the arms of the supporting piece the nucleus is situated (Pl. 18, figs. 3, 5). It is a rather large body measuring 10–16 μ and slightly oval in shape. On account of its close proximity to the deeply staining supporting pieces and the parabasals it is often difficult to obtain a clear picture of it unless the stain is greatly extracted from the rest of the body.

Bounding the nucleus there is a distinct membrane, immediately within which is a clear space measuring in a $10{\cdot}4\,\mu$ nucleus $2{\cdot}02\,\mu$ and in a $14{\cdot}1\,\mu$ nucleus $3{\cdot}4\,\mu$. This space is entirely devoid of threads radiating into it from the central chromatin mass, which is not homogeneous but composed of a ground substance, probably plastin, in which numerous chromatin masses are embedded. They are not, however, sharply defined bodies, but irregular as regards shape and size with indistinct margins merging imperceptibly into the ground-work (Pls. 18, 19, figs. 5, 7). Such is the characteristic "resting" nucleus.

(D) Axostyle.

The chief points regarding this structure I have already indicated, so that there only remains to describe certain details revealed by the employment of stains. Capriciousness regarding the reaction to stains is shown here as in

D. termitis, for in some slides the axostyle can scarcely be seen at all, while in others treated in the same manner, as far as can be ascertained, it is a prominent feature of the body. On the whole Dobell's hæmatein stain seems to be the best for demonstrating the axostyle, though it cannot be claimed as absolutely reliable for this purpose any more than the many other stains which I have tried. Also the method of fixing the preparation does not appear to affect the result. However, in slides showing the axostyle it appears to take origin in some of the basal granules of the supporting piece (Pl. 19, fig. 11). In its course through the body it runs ventral to the nucleus, but does not become attached in any way to the nuclear membrane.

While in the living animal it appears as a hyaline structure, very similar to that of D. termitis, when stained it is different. In D. termitis the axostyle retains its hyaline appearance, and a series of granules are seen running down the centre. In J. polytricha, on the other hand, the axostyle appears uniformly stained with no trace of median granules. Also it is much narrower in relation to the animal's size, measuring only 2μ in diameter, which is approximately the size of the axostyle of D. termitis, a much smaller animal.

Division.

(a) Possible Cause of Division.

The division stages of Jœnopsis are not readily found, which is in sharp contrast to what obtains in D. termitis, where every preparation contains a large number of animals in various stages of reproduction. For a long time I never encountered any specimen of J. polytricha in this phase of its life cycle, and I was beginning to despair of being able to work out the cycle of the animal when in April, 1918, a specimen in which the nucleus was in an early stage of division was encountered. The slide containing this form was made from the rectal contents of a male soldier termite. In the following month a female worker was found in which the parasites were reproducing themselves. In July and August, however, I obtained several slides from the sexes of all castes in which the protozoa were in what might be termed an epidemic of reproduction. Since that date until the end of December, when I had exhausted all my stock of termites, no other dividing forms were seen.

From the above it might be concluded that reproduction took place only during the warm months of the year, and that the higher temperature exerted some stimulus to reproduction in the parasites.

To test this hypothesis, during the months of October, November and December a few termites, representative of all castes, were placed in small boxes containing the wood on which they feed and incubated at a temperature of 90° F. The insects remained at this temperature for periods ranging from 3 days to 2 months. Throughout the entire period the insects remained in a perfectly healthy condition. Slides of their rectal contents were made at frequent intervals, usually twice a week, but in no case was there evidence of great reproductive activity. The table below gives the results of these experiments. It must be noted also that on killing the termites their protozoa appeared quite normal and in large numbers, showing that they had suffered no ill-effects from their changed conditions.

Table.

The word "negative" indicates that after examining the slides made of the rectal contents of the termite which had been killed no dividing Jœnopsis were found.

Soldier ♂,	killed after	3 days,	negative.
Nymph ♀	,,	,,	,,
,, ♂	,,	,,	,,
Worker ♀	,,	,,	,,
Soldier ♀	,,	5 ,,	,,
Nymph ♂	,,	7 ,,	,,
,, ♀	,,	7 ,,	,,
Worker ♂	,,	7 ,,	,,

Soldier ♂,	killed after	10	days,	negative.
Nymph ♀	„	14	„	„
Worker ♂	„	14	„	„
„ ♀	„	14	„	one dividing form.
Soldier ♀,	killed after	17	days,	negative.
Worker ♂	„	21	„	„
„ ♂	„	21	„	„
„ ♀	„	21	„	„
Nymph ♀,	killed after	25	days,	one dividing form.
„ ♂	„	28	„	negative.
Worker ♀	„	28	„	„
„ ♂	„	28	„	„
Soldier ♀,	killed after	32	days,	negative.
Worker ♀	„	35	„	„
„ ♂	„	35	„	„
„ ♂	„	38	„	„
Nymph ♂	„	56	„	three dividing forms.
„ ♀	„	56	„	negative.

These experiments are by no means exhaustive, but I had not sufficient material to carry out my investigations on a large scale or to determine the action of any external factor other than that of temperature. The results obtained, however, are not without interest, and seem to indicate that temperature is not the operative factor in bringing about reproduction. My view is rather that the conditions necessary are resident in the animals themselves and are more or less independent of other factors. The reason, then, for my finding reproducing animals in the warm months of the year and not in the cold I would ascribe, either to my not being able to examine sufficient material during these latter months, or to ill-luck in not killing animals which were harbouring parasites undergoing division, which phase possibly is rapidly passed through.

(B) Division Phases.

The early stages of division are exceedingly difficult to find, and even in those slides in which the majority of the animals are reproducing it is almost impossible to find a specimen showing the method by which the body organella become reduplicated. That they do so, however, at an early stage is evidenced by the many animals seen with a double set of these structures, but with the nucleus in the various stages of division (Pl. 19, figs. 10, 15). It is probable that the supporting piece of the head and the parabasal threads undergo longitudinal division. My positive evidence for this view is based on one animal only, of which (Pl. 19, fig. 9) is a drawing. Here the parabasal threads and supporting piece are divided, or just about to complete their longitudinal division. Support for the conclusion is also given by the fact that in all reproducing animals—at whatever stage—a double set of organella can be seen (Pls. 19, 20, figs. 10, 15, 16, 17, 20). If they had been produced as new outgrowth and not by division of the preexisting structures one would expect to find the intermediate growth stages, as was the case in the chromatic base of the undulating membrane of D. termitis. Such stages have never been observed.

As regards the rod-like parabasal bodies situated on the threads I am unable to state their method of division. There is no evidence of their breaking into granules, which are subsequently absorbed as Grassi has described (**6**, **7**) in Jœnia annectans.

Turning now to the nucleus the first stage of division consists in the resolution of the chromatin into a large number of round granules, the number of which appears to be indefinite, but ranging between 50 and 60. The plastin framework is also broken up into a cloud of tiny granules distributed throughout the whole nucleus (Pl. 19, fig. 11). Coincident with this process the nucleus increases in volume, reaching a diameter of 7·7 μ.

The next stage consists in the aggregation of the granules to form thin rods whose number varies from 8 to 12 (Pl. 19, fig. 12).

These rods now separate one from another, an equal number passing to each end of the elongating nucleus in which no trace of spindle-fibres can be detected. A constriction in the membrane and further elongation of the nucleus occur, and at the same time the plastin granules aggregate round the chromatin rods (Pl. 19, figs. 10, 13, 14).

Finally complete nuclear constriction takes place and the two daughter-nuclei remain connected together by a thin thread (Pl. 19, fig. 15).

At this stage the rods break into round bodies, variable in numbers, which become irregularly distributed through the nuclear substance (Pls. 19, 20, figs. 16, 17, 18, 19, 20). These bodies rapidly lose their individuality and the typical resting type of nucleus is formed.

A curious feature of the whole process is that one of the daughter-nuclei is usually a little further advanced in reconstruction than the other one (Pls. 19, 20, figs. 15, 17, 20).

The plastin component of the nucleus aggregates in the middle at an early stage in division (Pl. 19, fig. 13), and during the changes described above it becomes approximately equally distributed between the two nuclei, forming finally the ground-work of the new bodies (Pls. 19, 20, figs. 14–20). As the nuclei migrate one from the other the thread connecting them disappears, but the two independent nuclei still continue to separate. The organella of the body, already divided, follow the nuclei; thus finally two complete animals are produced only requiring that the plasma should constrict in order for them to lead an independent existence (Pl. 20, fig. 20).

A characteristic feature of the reproduction is that during the nuclear migration the organella undergo a rotation through 180°. During the early division phases they are similarly orientated (Pl. 19, fig. 10), but later rotation occurs as is shown in Pls. 19, 20, figs. 15, 16, 17, 20. It is of interest that Grassi found the same to obtain in Jœnia annectans (**6**, p. 251). "I due nuclei, le due aree flagellate con relative regoli, collari, ecc, quando i due individui stanno per separarsi, in molti casi, forse in tutti, si sono attontanti l'uno dall' altro di 180°."

As the supporting pieces separate each carries with it a portion of the striations and body flagella, so that in animals in this condition (Pl. 19, fig. 15) each half of the complex possesses about half the total number of striations, leaving the other half of the body naked.

It is probable that after the separation of the animals into two, the portion of the body devoid of striations forms them "de novo." It will have been noticed that during the nuclear division no division centre of any kind is formed, and there is no trace of blepharoplasts, centrioles or even the paradesmose found in D. termitis—in fact the whole process is an example of a simple mitotic division.

There remains to be described the origin of the daughter-axostyles. As I have pointed out in a previous work (**2**), the accounts of the formation of these structures are widely divergent from one another, and are impossible to reconcile unless one assumes that this organ in the various flagellates has different origins.

As there is no extra-nuclear spindle in Jœnopsis it is obvious that the daughter axostyles cannot be formed from such a structure as has been described in other flagellates.

For D. termitis I gave reasons for believing that the axostyle underwent longitudinal division, and showed conclusively that the paradesmose could have no connection with it. This view is strengthened by the investigations in Jœnopsis.

Whether, however, the new axostyles are derived from the existing one by splitting or whether they are developed "de novo" by growth from basal granules, as described by Kuczynski, I am unable to state with certainty.

Unfortunately an animal was never encountered in which the axostyles showed signs of division, but on the other hand there is no trace in any of the preparations of development by growth. In animals which have almost completed their reproductive phases two similar axostyles are always seen (Pl. 19, fig. 16). I therefore incline to the view that in Jœnopsis, as in D. termitis, there is a rapid longitudinal division of the old structure.

Cyst-Formation and Sexual Process.

Neither-cyst formation nor sexual process can be found in J. polytricha—a result which accords with previous investigation on termite protoza. Probably Grassi supplied the reason for this when in 1911 he wrote: "Probalimenti la soppressioni dell'incistamento e for'anche quella della fecondazione sono rapportabili al costume or ricordato dei Termiti di mangiar la feccia dei propre compagni all'atto della emissione."

General Considerations.

(a) Classification.

At the outset I must apologise for adding one more variation to the name Jœnia, which has already suffered much change at the hands of other workers. Thus we have Microjœnia, Mesojœnia, Jœnina and Staurojœnina founded by Grassi, to which Janicki (**11**) had added the genus Parajœnia. It may appear that I introduce unnecessary confusion to the literature by creating the genus Jœnopsis, but this name seems to me to express more clearly than any other the superficial resemblance that Jœnopsis polytricha has with Jœnia annectans. Grassi's (**7**) definition of the genus Jœnia is as follows: "Zona di flagelli richiamente la figure del nostro cuojo capelluto: pero non esattemente simmetrico ai due lati: bastoncello assile a mestolo, terminante posteriormente a oliva: doppio collare con figlioline: lamina e nastrino lettera esse evidentissimi, ciocondati da speciale lacune a piene di liquido: forme molto grandi."

From the above it is clear that Jœnopsis is in broad outlines similar to Jœnia. Thus in both forms there is a tuft of flagella at the "head" end of the body, which arises from a supporting piece in Jœnopsis and from a flagellate area, crossed by transverse and longitudinal striations in Jœnia. In both animals there is a well-developed axostyle, though simpler in Jœnopsis, for there is no trace of the "mestolo" or "oliva" of Jœnia.

Probably, however, the most characteristic feature common to the two genera is the well-developed parabasal apparatus, consisting of parabasal threads to which the parabasal bodies (figlioline) are attached, the whole complex in J. polytricha strongly recalling the description given by Grassi of the "regolo," "collare" and "figlioline."

The quadrangular areas of protoplasm at the anterior end of Jœnia, termed by Grassi "lamina e nastrino a lettera," I have been quite unable to find in Jœnopsis, whose anterior protoplasm is perfectly homogeneous, and in this respect more nearly resembles the genus Mesojœnia where the structures are poorly developed. Further, there is no evidence in Jœnopsis of a lake filled with fluid such as described in Jœnia. Turning to other characters which separate Jœnopsis from Jœnia, its neighbouring genus, there may be mentioned the groove of the body. Of greater interest, however, are the longitudinal striations, with their attendant flagella of which I have written above.

The supporting piece of the head is also a characteristic feature, not found in Jœnia, but represented in the recently described genus, Staurojœnina, by a complicated body divided into four parts from which the anterior flagella arise. Jœnopsis and Staurojœnina are similar in lacking the definite blepharoplasts (batacchio) found in Jœnia. The genus Parajœnia (Janicki) appears to be widely separated from those mentioned above. The flagella are arranged in two curved lines at the anterior end, there is a trailing flagellum arising from a basal granule (blepharoplast), and the parabasal apparatus consists of two curved bodies each with its parabasal thread attached to the granule from which the trailing flagellum springs.

I would place Jœnopsis near to the genera Jœnia and Mesojœnia with affinities with Staurojœnina, but distantly connected with Parajœnia.

Using the classification of Grassi the family Jœniidæ would be composed of the following genera:

(1) Jœnia (Grassi).
(2) Mesojœnia (Grassi).
(3) Microjœnia „
(4) Jœnia „
(5) Staurojœnina „
(6) Jœnopsis (Cutler).
(7) Parajœnia (Janicki).

(B) Parabasals.

In Jœnia Grassi has described a "regolo" and "collare" to which are attached small rods—the "figlioline. Janicki, to whom we owe the term "parabasal," homologises the "regolo" with the parabasal thread and the "collare" with its attached rods, as parabasal bodies. For the reasons given by Janicki I employ his nomenclature rather than that of Grassi. As I have pointed out already the parabasal apparatus of Jœnopsis is essentially similar to that of Jœnia, and I have therefore called the two long bands arising from the supporting piece the parabasal threads and the attached rods the parabasal bodies.

There are, however, points of difference between the structures in Jœnia and Jœnopsis, for in the former there is but a single parabasal thread which divides at its apex into two bands, one going to the blepharoplast and the other to the margin of the basal lamella of the flagella. The thread-like portion of the "collare" in Jœnia is but the continuation of the parabasal thread surrounding the axostyle. On this encircling band the parabasal bodies are attached. Thus Grassi includes in his term "collare" the parabasal bodies and the posterior part of the parabasal thread.

In Jœnopsis there are two threads, arising from the supporting piece. It seems probable that we have in Jœnopsis a condition more simple, for if the distal ends of the parabasal threads were joined together they would encircle the axostyle, and a further fusion of the proximal ends of the threads into a single band would produce a condition similar to that found in Jœnia. Further, the division of the band at the anterior ends in this animal renders it probable that it is in reality composed of two threads fused together.

As regards the division of the parabasal bodies Grassi stated in his early paper (**4**) that they were reduplicated after the formation of the nuclear spindle, but was not certain whether they were developed "de novo" or by division, though he inclined to the latter view. In his later work (**6**) he described the bodies as usually being broken down into granules, which were absorbed and a new set of rods developed. In some cases, however, this does not occur, the whole apparatus being passed over to the daughter-organism and a new one developed in the old animal. As I have already stated the evidence in Jœnopsis is in favour of the view that the daughter parabasal bodies are produced by longitudinal division of the old ones as occurs with the parabasal threads.

In my earlier paper I have given a short account of the various modes of formation of the parabasal apparatus in the different genera of termite parasites; there only remains for me to point out that in Parajœnia the structures are very different in formation from what obtains in Jœnia, Mesojœnia and Jœnopsis, but that these three genera, on the other hand, broadly agree with the genus Lophomonas.

(c) Blepharoplast and Nuclear Division.

In both Jœnia and Parajœnia there is a well-marked body from which the parabasal threads arise together with the training flagellum in Parajœnia and the "sospensorio," etc., in Jœnia. In Jœnopsis no such body is found.

The chief interest in the nuclear division is the non-existence of any spindle structure, which is of importance in connection with the development of the axostyle. In my previous paper (**2**) reasons are given for doubting the view that the axostyles were always developed from the spindle, and J. polytricha supplies a further proof for my contention. Comparing the nuclear division of Jœnopsis with that of Jœnia it is at once evident that we are dealing with a simpler type. In the latter animal there is a well-developed spindle, whose origin is not determined, but which ultimately takes up a position in a notch of the nuclear membrane. Elongation of the spindle occurs, and with it the chromatin is resolved into a skein, which splits transversely. Finally the nucleus divides and the daughter-bodies are attached for some time to the ends of the spindle. By further elongation the spindle becomes U-shaped, extending through the body, until in the end it splits into two to form a portion of the new axostyles.

It is obvious that as regards nuclear division there are few or no points of agreement between the two genera; and on account of the simpler nature of division, together with other points mentioned in the earlier part of the paper, I am inclined to regard Jœnopsis as more primitive than Jœnia.

Diagnosis of Jœnopsis polytricha.

A brief diagnosis of J. polytricha will be of use to students of termite protozoa and at the same time serve as a summary to this paper.

Jœnopsis polytricha, n. gen. n. sp.—Flagellate of large size, the average being 112 $\mu \times 62{\cdot}3\ \mu$. The animal is divided into two regions by a groove running somewhat obliquely round the body, thus demarcating a smaller anterior end from a larger posterior one. No cytostome is present. At the anterior end of the body there is found a supporting piece, with numerous basal granules from each of which a flagellum arises, the whole complex of flagella being arranged in a tuft, forming a whip-like locomotor apparatus.

The anterior two-thirds of the body is traversed by striations, arranged in a right- and left-handed series, taking origin in the supporting piece. These striations represent the edges of raised areas of the body surface, on which areas are situated basal granules, each with a flagellum.

Two parabasal threads, on which are developed numerous parabasal bodies, arise from the supporting piece, as does also a well-developed axostyle. The plasma is commonly granular and not differentiated into ectoplasm and endoplasm.

The nucleus is found between the two arms of the supporting piece, and is large, measuring from 10–16 μ in diameter. It possesses a well developed membrane with chromatin in the forms of irregular bodies embedded in a plastin groundwork. A clear space is seen between the chromatin and the peripheral membrane.

The method of reproduction is by simple division into two; no cyst-formation or sexual process has been observed.

The first stage of division is the reduplication of the supporting piece and parabasal apparatus; this probably takes place by longitudinal division of the existing bodies.

The nucleus divides by a simple mitosis with no development of spindle fibres or centrioles. Further, there is no formation of an extra spindle or paradesmose.

The origin of the daughter-axostyles is not clear, but the evidence points to the conclusion that the old axostyle divides logitudinally.

Habitat. Hind gut of the termite Archotermopsis wrougtoni Desn.

Food. Particles of wood and cellulose.

JŒNOPSIS CEPHALOTRICHA, N. SP.

In the living condition it is at once apparent that this animal resembles J. polytricha in many respects, and I think that Imms (**11**) has included this species in his description of Species β. This is, however, incorrect, for J. cephalotricha lacks the body flagella of J. polytricha; and other characters, to be described below, show it to be a distinct species.

At the anterior end of the body there is a tuft of flagella (Pl. 20, figs. 21, 22 *H.F.*) which serve as locomotor appendages.

As in J. polytricha, the endoplasm contains a number of wood particles which are confined to the posterior end of the body. No sharp line of demarcation can be found between the ectoplasm and endoplasm, which is granular, though alveoli are by no means rare.

I have been unable to find a cytostome or the method by which food is ingested.

The groove so characteristic of J. polytricha is not developed, but the anterior end of the body is raised into a broad papilla-like process whose origin is probably similar to that of the same structure in J. polytricha.

As regards movement there is a difference between the two species: in J. polytricha progression is by a kind of gliding motion with continual revolution, due to the head flagella functioning to one side of the body. In J. cephalotricha the flagella alternate in their action from one side to the other, so that although the gliding movement is preserved there is no longitudinal revolution, but a slight oscillation from side to side.

Morphology.—J. cephalotricha is a smaller animal than J. polytricha, its length varying from 85–90 μ with a width of 45–60 μ, the average size being 87 μ × 51 μ. Thus the shape is more or less oval with rounded ends.

At the anterior extremity there is a supporting piece (Pl. 20, figs. 21, 22), which shows the same reaction to stains as did the same structure in J. polytricha, and on each side there are numerous basal granules from which the flagella arise (Pl. 20, fig. 22).

From this supporting piece striations are given off which traverse about one-third of the body, and are developed all round it and not in a right- and left-handed series (Pl. 21, fig. 23).

Also these striations carry no basal granules or flagella. In J. polytricha the striations represent the edges of "hills" on which basal granules are placed, and every pair of striations joins together at the anterior and posterior ends of the body. In J. cephalotricha, on the other hand, the striations are simply thickenings of the body surface—in fact are comparable to those developed in the body of Polymastix. Probably they are strengthening structures and of totally different origin from those of J. polytricha.

Parabasal bodies and threads are not developed, but an axostyle, similar to that of P. polytricha, takes origin in the supporting piece (Pls. 20, 21, figs. 21, 22, 24, 25).

The nucleus is situated at the anterior end of the body and is bounded by a distinct membrane, inside of which large chromatin masses are embedded in plastin. The diameter of the nucleus is about 5·7 μ (Pl. 20, fig. 22).

Reproduction.—Unfortunately I have been unable to trace the life-cycle in this animal, though I have searched through the whole of my material. The only stages found are figured in Pl. 21, figs. 24, 25. In both these animals there are two nuclei, which have undergone reconstruction, and two supporting pieces with their flagella. This indicates that probably the reproduction is broadly similar to that of J. polytricha. There is no trace of any spindle structure, but this is not evidence that such a body does not occur—though by analogy with J. polytricha it is improbable—for, if formed, it may easily have disappeared as the stages in question are undoubtedly late ones.

MICROJŒNIA AXOSTYLIS, N. SP.

This animal is quite distinct from the two already described but is closely related to Microjœnia hexamitoides (Grassi).

The size is smaller than that of J. cephalotricha, ranging from 16·16–30 μ in length, and 12·1–25 μ in width.

At the anterior end there is a tuft of flagella arising from a series of granules arranged in two or three longitudinal rows (Pl. 21, figs. 26, 27).

The extreme anterior end of the body is not raised into a papilla, but is much thickened and flattened. A similar development is described by Grassi (**6**, **7**), and he likens it to an operculum of a lake containing fluid. I have no evidence of the presence of any fluid in this region of the body; on the other hand I believe this thickening is an adaptation on the part of the animal to the strain produced by the vigorously functioning flagella. Whereas in Jœnopsis this strain was compensated by the supporting piece, here in M. axostylis no such support is developed, but instead the ectoplasm is flat and thick.

The surface of the body is smooth with no trace of striations. The most distinguishable feature of the animal is the large axostyle arising from the anterior end of the body; and which, passing ventral to the nucleus, pierces the posterior end of the body surface. Its most noticeable feature is the width, which is 4·5–5 μ, and which, relative to the body width, is enormous. Also it is hyaline in appearance with no affinity for stains, in this respect resembling the axostyle of D. termitis.

Unfortunately I have been unable to find any reproductive phases. Grassi (**6**) defines the genus Microjœnia as follows:

"Flagelli sorgenti in fitte serie sub-longitudinali da una zona vicina all'extremità anteriore delimitante perciò quivi un cerchio anteriore, il quale si presenta come l'operculo di un augusta lacuna contenente liquido, e risalta perchè sprovisto di flagelli: bastoncello assile: una serie di speciali corpi tondeggianti al margine posteriore della zona suddetta: fore piuttosto piccole."

It is obvious from the above that the animal I have described should be placed as a new species in the genus Microjœnia.

In conclusion I wish to express my thanks to Mr. J. B. Robinson for re-drawing for publication the figures which illustrate Pls. 18, 19, 20, 21, and to the Zoological Department, Manchester University, for a grant which enabled this work to be carried out.

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The papers marked * contain further references to the literature of Termite parasites.

EXPLANATION OF PLATES 18 TO 21,

Illustrating Mr. D. Ward Cutler's paper on "Protozoa parasitic in Termites."

[All the figures, except figs. 1 and 2, are drawn from fixed and stained preparations. The optical apparatus employed was as follows: Zeiss apochromatic oil-immersion objective 2 mm. (N.A. 1·3) and compensating oculars 6, 12, 18. Critical illumination was always used. The method of fixation and staining and the appropriate magnification is given in the case of each figure, except figs. 1 and 2.

The following abbrevations are employed: S.A. Schaudinn's sublimate-alcohol mixture. S.A. (D.J.) Schaudinn's sublimate-alcohol as modified by Dobell and Jepps. Fl. (Gat.) Fleming's strong fluid as modified by Gatenby. H.I.H. Heidenhain's iron-alum hæmatoxylin. D.H. Dobell's iron-hæmatein.

The lettering of the figures on the plates is as follows: *A.X.* Axostyle. *B.F.* Body flagella. *B.G.* Basal granules. *Fl.* Flagella. *G.R.* Groove. *H.F.* Head flagella. *P.B.* Parabasal body. *P.T.* Parabasal thread. *S.P.* Supporting piece. *S.T.* Striations.]

PLATE 18.

Fig. 1.—Side view of living Jœnopsis polytricha. Note the supporting piece, parabasal thread and axostyle. The striations do not appear in a right- and left-handed series as in the face view of the animal seen in fig. 2.

Fig. 2.—Freehand drawing, as in fig. 1, of a living animal seen in face view, showing the right- and left-handed arrangement of the striations, also the groove dividing the animal into "head" and "body" regions. The flagella in the living animal are more numerous than represented in the drawing.

Fig. 3.—Stained preparation of J. polytricha, showing the supporting piece, parabasal threads and axostyle arising from the supporting piece. The basal granules and flagella are omitted from the drawing. Parabasal bodies are not seen owing to the method of fixation. × 450. S.A., D.H.

Fig. 4.—Highly magnified drawing of two striations with the basal granules and flagella. × 1800. Fl. (Gat.), H.I.H.

Fig. 5.—Head region of J. polytricha with supporting piece and its basal granules (flagella omitted). Parabasal threads and bodies seen. Note the resting nucleus with its chromatin bodies embedded in the plastin ground-work. × 950. Fl. (Gat.), H.I.H.

Fig. 6.—Highly magnified drawing of a parabasal thread with its attached parabasal bodies. × 1000. Fl. (Gat.), H.I.H.

PLATE 19.

Fig. 7.—Resting nucleus of J. polytricha. × 950. S.A., D.H.

Fig. 8.—Section through the body of J. polytricha, showing the

folded surface (striations) and basal granules. × 1000. S.A. (D.J.). H.I.H.

Fig. 9.—"Head" region of animal showing what is probably the first stage of division. The organella are in most cases reduplicated, but the nucleus is still in the resting condition. × 950. Fl. (Gat.), D.H.

Fig. 10.—"Head" region with dividing nucleus and double set of organella. Parabasal bodies are not seen due to method of fixation. × 950. S.A. (D.J.), D.H.

Fig. 11.—Anterior end of animal with supporting piece and basal granules (flagella omitted). The basal granules on the striations are seen, but the anterior ends of the striations are omitted. Note axostyle arising from the supporting piece. Nucleus in the first stage of division with the chromatin resolved into granules. × 950. S.A. (D.J.), H.I.H.

Fig. 12.—Nucleus with its so-called chromosomes; plastin in the form of granules. × 1800. S.A., H.I.H.

Fig. 13.—Dividing nucleus with chromatin aggregated at each pole and the plastin situated at equator. No spindle-fibres or centrioles present. × 950. S.A. (D.J.), H.I.H.

Fig. 14.—Dividing nucleus in which membrane is constricting. × 950. S.A. (D.J.), H.I.H.

Fig. 15.—Dividing J. polytricha in which two sets of organella are seen. At the top of the animal the middle portion of the striations are seen passing over the edge of the body. Axostyles omitted. × 450. On the right of the animal the nuclei, more highly magnified, are drawn. Note that the upper daughter-nucleus is in a more advanced stage of reconstruction than the lower one. × 950. Fl. (Gat.), H.I.H.

Fig. 16.—Animal at the close of division; double set of organella, with the exception of parabasal bodies. Striations and flagella omitted. × 450. The daughter-nuclei, more highly magnified, are seen below. × 950. S.A. (D.J.), H.I.H.

PLATE 20.

Fig. 17.—Dividing J. polytricha in which the parabasal bodies and axostyles are not visible. × 450. As in the previous figures a more highly magnified drawing of the nuclei is seen to the right. The lower nucleus is more advanced in the reconstruction process than the upper one. × 950. S.A. (D.J.), D.H.

Fig. 18.—Daughter-nuclei directly after separation. × 1800. S.A. (D.J.), H.I.H.

Fig. 19.—A daughter-nucleus at the close of division. Note plastin

granules and the large chromatin bodies, approximately thirty-two in number. × 1800. Fl. (Gat.), H.I.H.

Fig. 20.—Animal near the close of reproduction. × 450. The daughter-nuclei below are magnified. × 950. Fl. (Gat.), H.I.H.

Fig. 21.—Drawing of a specimen of Jœnopsis cephalotricha. Note supporting piece with striations and axostyle. Nucleus at anterior end probably in the first stage of division. × 950. Fl. (Gat.), H.I.H.

Fig. 22.—As above, but nucleus in resting stage; basal granules are seen on the supporting piece. × 953. S.A., H.I.H.

PLATE 21.

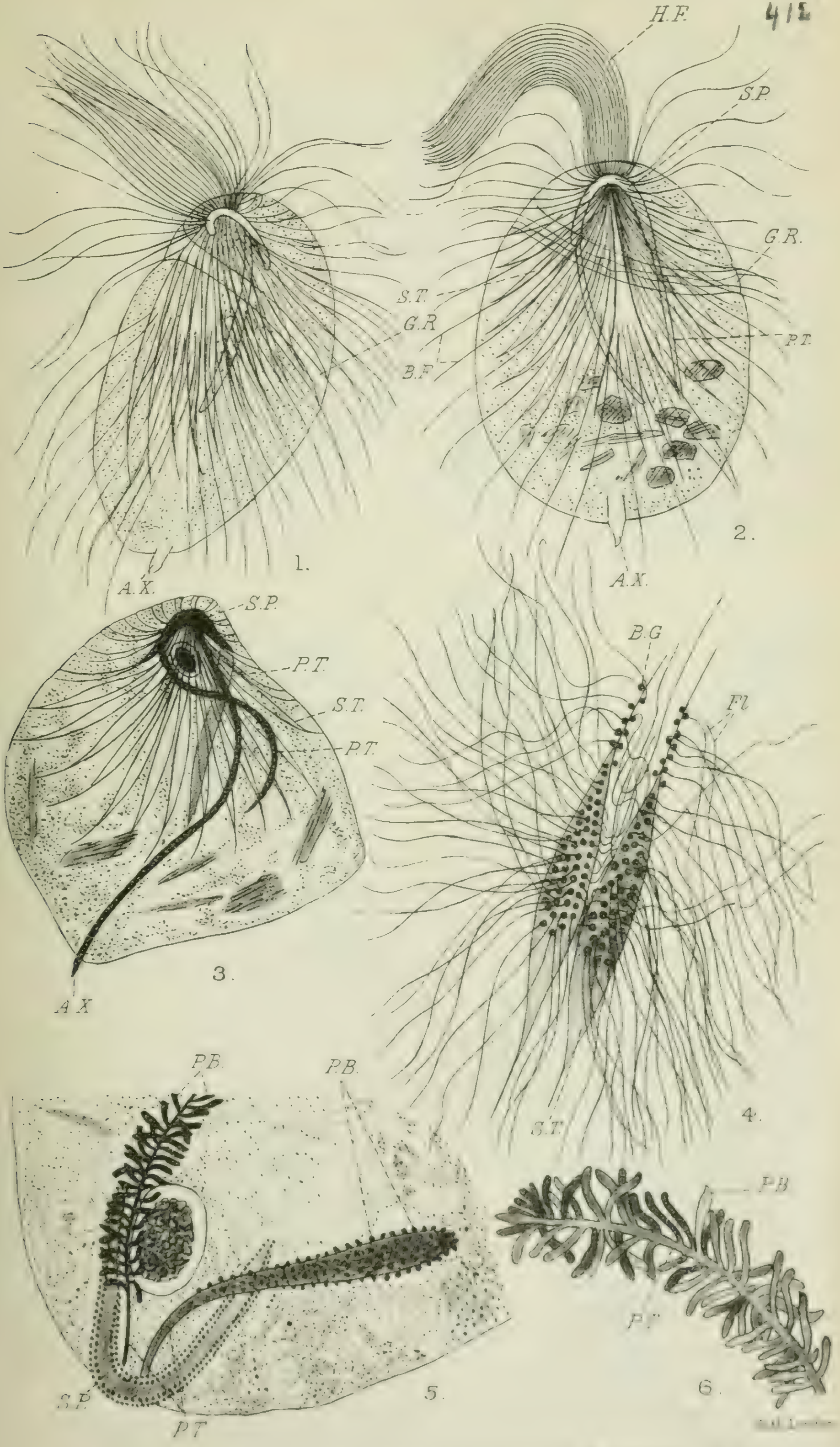
Fig. 23.—J. cephalotricha seen from above. The striations are observed radiating from the supporting piece and the nucleus between the arms. × 950. S.A. (D.J.), H.I.H.

Fig. 24.—Animal at the end of division process, with two supporting pieces and axostyles. × 950. Fl. (Gat.), H.I.H.

Fig. 25.—As above. × 950. S.A., D.H.

Fig. 26.—Stained specimen of Microjœnia axostylis. Note the broad axostyle arising from the anterior extremity, also the thickened anterior border with basal granules and flagella. × 950. S.A., D.H.

Fig. 27.—As above. × 950. Fl. (Gat.) H.I.H.



CUTLER—PROTOZOA PARASITIC IN TERMITES, Pt 2.

Quart. Journ. Micr. Sci. Vol. 64, N.S. Pl. 19.

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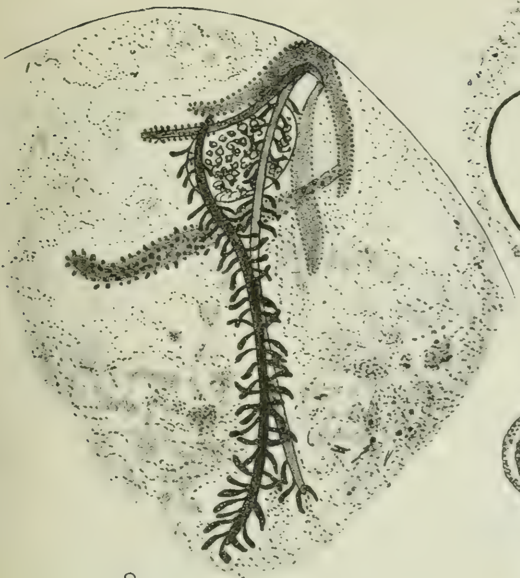

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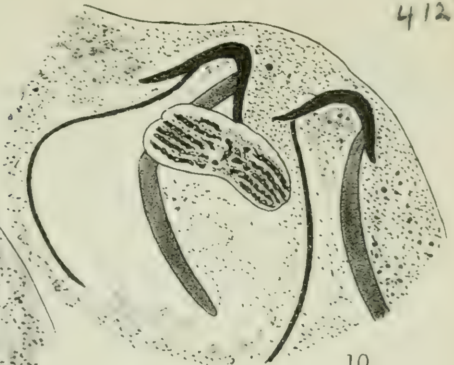

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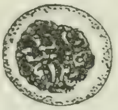

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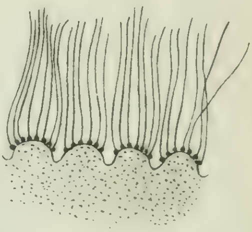

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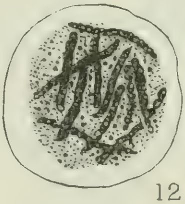

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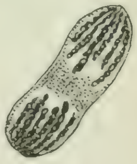

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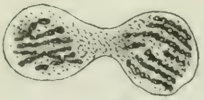

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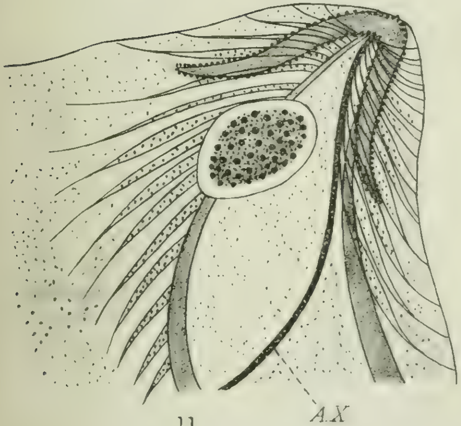

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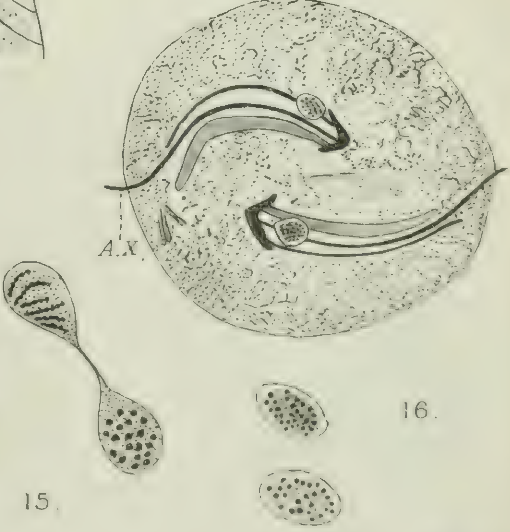

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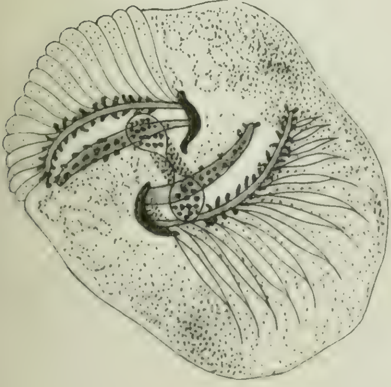

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CUTLER — PROTOZOA PARASITIC IN TERMITES, Pt. 2.

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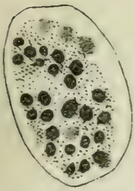

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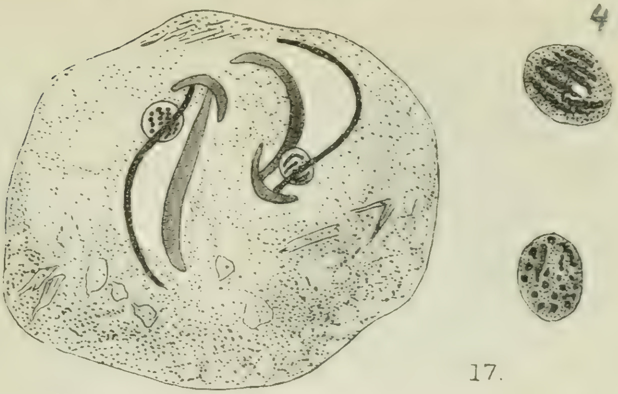

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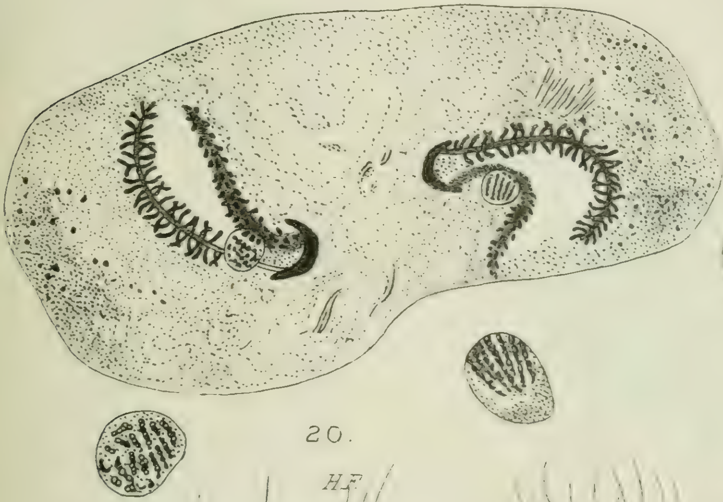

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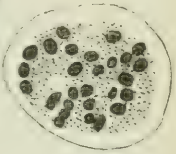

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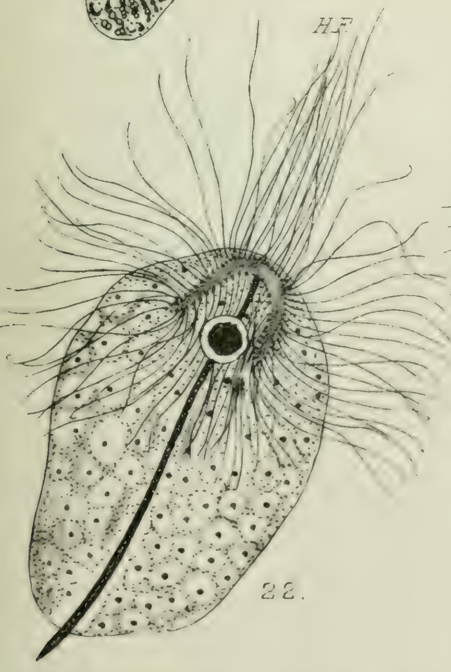

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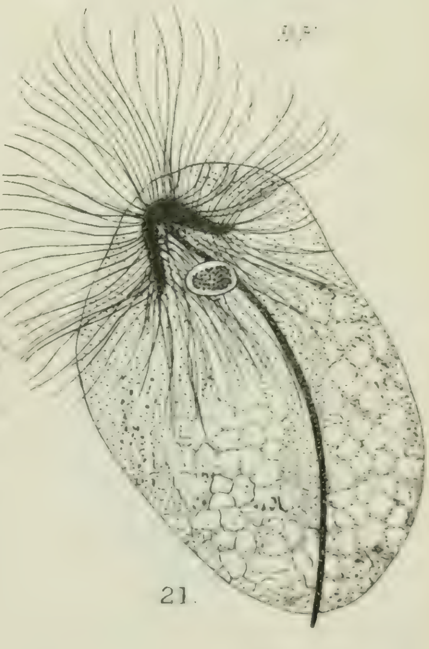

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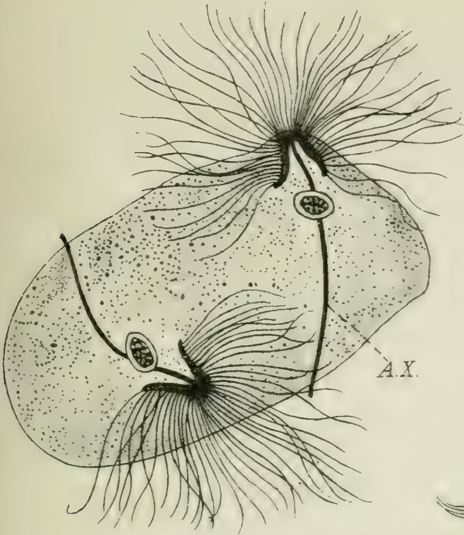

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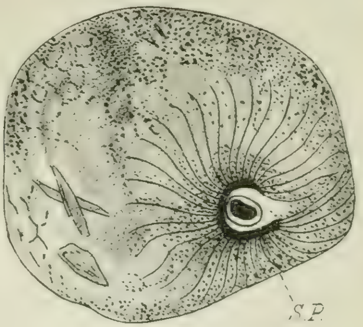

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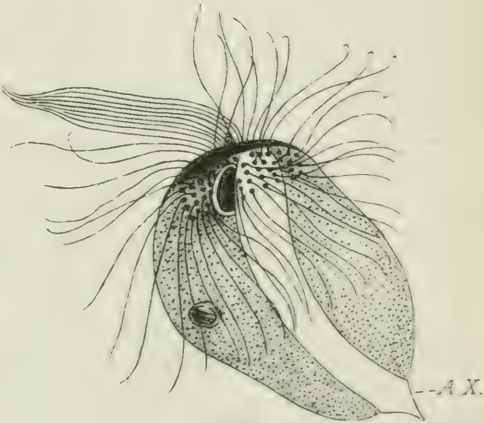

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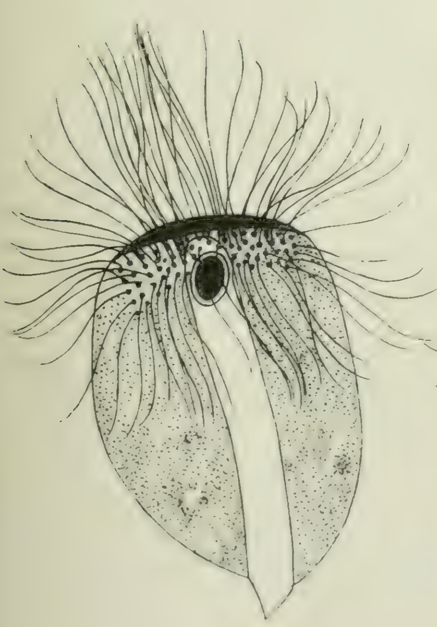

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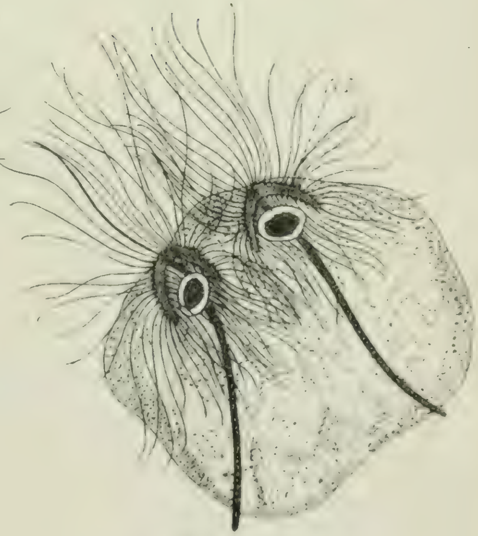

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CUTLER—PROTOZOA PARASITIC IN TERMITES. Pt 2.

Note on an Amœba-like Parasite from Clavellina.

By

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With 1 Text-figure.

In the summer of 1910 at Naples I noticed that the stomach of small (and therefore semi-transparent) individuals of the social Ascidian, Clavellina lepadiformis, sometimes contained a number of solid objects. When this was so it was obvious, because the ciliary action in the stomach kept these objects in constant and rapid motion.

On investigation the objects in question proved to be small parasitic amœbæ, of a species which does not appear to have been previously described. It is proposed to give it the name Amœba clavellinæ.

General Description.—The amœbæ are rather small, the average diameter being 14–15 μ, with occasional giants of 18–17 μ and a few considerably smaller of 11–12 μ diameter. They were usually subspherical, but occasionally ovoid, the long diameter then averaging 17–18 μ. As they appear never to adhere to the walls of the stomach, but to be whirled about passively in the nutritive fluid of the lumen, we should not expect to find any great development of pseudopodia; and, as a matter of fact, these were never observed. I did not notice anything but the mildest irregularities of contour, and even the change to the ovoid shape was exceptional.

Cytoplasm.—In one respect this amœba is remarkable. Although in perhaps the majority of specimens there is a definite ectoplasm, marked off from the endoplasm by a definite sharp contour (Text-fig. A and B), yet in some there may be no ectoplasm whatever, the darker, densely granular and more refringent endoplasm coming right up to the edge of the cell. A few specimens are intermediate, in that, although both layers are present, there is no sharp demarcation between them; and one of this type was seen in which the ectoplasm was absent over *part* of the body. The endoplasm appears practically homogeneous in ground-substance, with quite large, round or ovoid granules scattered through it. No contractile vacuole was observed. In one stained specimen a vague, somewhat clearer space was seen close to the nucleus.

Nucleus.—This was almost always rather excentric in position. It was almost spherical, varying in diameter from about 4 to 5 μ, while the nucleolus, as I shall call the central body, varied from 2 to $2\frac{3}{4}\,\mu$ in long diameter, being often rather more ovoid than the nucleus itself.

The nucleus, like the cytoplasm, varied considerably in structure. The following may be taken as its typical plan (see Text-fig. C, as an example): On the outside there is a definite, double-contoured, dark-staining nuclear membrane (*nu.m.*) Next comes a clear ring, which I shall call the first clear band, or, for brevity's sake, *c.* 1; then another dark band, usually thicker than the nuclear membrane—the granular band (*gr.b.*); then a second clear band (*c.* 2); and finally, a large dark nucleolus (*ncl.*), with a sharp edge, usually containing one or more clearer spaces towards its centre.

In the commonest form the granular band is fairly sharply defined, composed of very fine granules, and uniform in thickness (and about twice as thick as the nuclear membrane), while the clear bands are quite clear and do not take up staining reagents. Each is about as thick as the nuclear membrane. The nuclear membrane usually stains deepest, then the nucleolus, then the granular band.

The following variations may occur, while the general type is preserved. The granular band varies—(1) In size: it may be only as thick as the nuclear membrane, or up to nearly three times as thick. (2) In consistency: sometimes it appears absolutely structureless (Text-fig. c), sometimes homogeneously granular, the granules of various degrees of coarseness; sometimes it loses its homogeneous character, consisting either of a faint structureless ground-substance with scattered dark granules embedded in it, or broken up altogether into more or less irregular large lumps. (3) In shape, being sometimes very thick in one part, and so resembling a signet-ring.

TEXT-FIG.

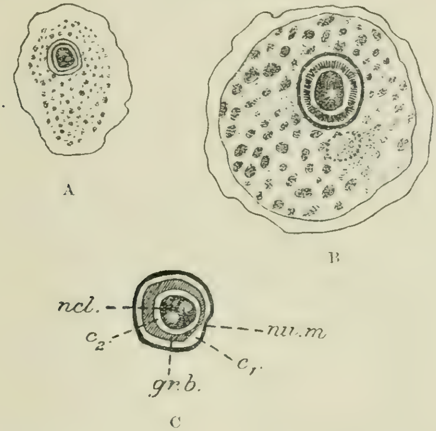

FIG. A.—An amœba with broad, clearly-defined ectoplasm. Nucleus of typical plan, with very thin granular band. Some clearer ovoid spaces in the nucleolus. The animal has elongated to an oval shape. FIG. B.—Amœba with thinner but very sharply marked-off ectoplasm. Nucleus of typical plan, the granular band of medium breadth, finely grained. One vague clear space in the nucleolus. FIG. C.—Nucleus of another specimen. *ncl.* the nucleolus; *nu.m.* the nuclear membrane; *c.* 1 and *c.* 2 very clear; the "granular band" (*gr.b.*) quite homogeneous, thin at one side. Two round clear spaces in the nucleolus.

There are further variations in the staining properties of the nucleus. The relative tingibility of the parts is usually retained, although occasionally the granular band exceeds the nucleolus in depth of staining; but occasionally either the nucleolus or the granular band (or both) is exceptionally pale.

The other main type of nucleus differs in possessing no outer clear band, the granular band being broader and coming right up to the membrane. Intermediate forms, however, exist, since stages in the differentiation of the clear band can be distinctly seen. In some specimens there is no indication of it whatever, the broad granular band being homogeneous in texture throughout its breadth. In other specimens the granular band is structureless and rather pale, with a row of few and small granules round its inner edge. In others these granules are more numerous, in others, again, larger. Finally one specimen was seen in which the typical plan was completely realised, save that the outer clear band was just faintly tinged with the stain.

What the meaning of these variations may be is at present quite uncertain. In two specimens no trace of a nucleus could be seen; whether the chromatin was in the form of chromidia, and lost to view among the cytoplasmic granules, could not be ascertained.

Habitat and Life-history.—I never saw the amœba anywhere save in the stomach of the ascidian. No cysts were ever observed, nor any indications as to further stages in the life-history. They presumably divide by binary fission in this vegetative stage, as is indicated by this difference in size, but no mitotic figures were ever observed. Although I had been working at Clavellina since January, I did not notice the amœbæ until April. Whether this was merely an oversight, or related to some fact of their life-history, I cannot say. The amœbæ may be so numerous as almost to fill the stomach.

In one respect the parasite is very remarkable—in the incessant and very speedy motion in which it spends the whole of its vegetative existence.

The life-histories of amœbæ are none too well known, of marine Amœbæ especially so. I have not the opportunity myself of continuing my observations, and am therefore publishing these fragmentary notes in the hope that some protozoologist may thereby be incited to attack the problem of the life-history of amœba in a parasitic form which not only inhabits a common and easily-kept host, but has the rare property of being visible, *in vivo*, in its natural habitat.

December, 1919.

On the Occurrence of Protohydra in England.

By

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The Protohydra leuckartii is a minute, solitary hydrozoon (about 3 mm. in length when fully extended), entirely devoid of tentacles and known to increase rapidly in numbers by simple transverse fission.

It was discovered fifty-two years ago by Greeff (**1**) in an oyster park at Ostend. For twenty years after its original discovery it was searched for in vain, but at last, in the autumn of 1891 and the spring of 1892 (fide Chun (**2**)), it was rediscovered in the same locality. There is no record of the occurrence of Protohydra in any other locality than Ostend, nor is there any record, so far as I am aware, of its occurrence at Ostend since the spring of 1892.

Four years ago, however, my friend Mr. Herbert Ashby found Protohydra leuckartii in great abundance in the pools on the tidal marshes of the river Hamble near Southampton, and thanks to his kindness and scientific spirit I have had the opportunity of observing it alive and studying its structure during several months of each succeeding year.

Our present knowledge of Protohydra is based on the observations recorded by Greeff, and the more detailed accounts of the minute structure of specimens, collected and preserved by Greeff, which have been published by Chun (**2**) and Aders (**3**). But notwithstanding the careful investigation of the material at their disposal by these three observers we are still in complete ignorance of the sexual method of reproduction in Protohydra, and it has been in the hope of discovering how and when this important little hydrozoon produces its ova and spermatozoon that Mr. Ashby and the present writer have continued our observations during the past four years.

This paper is, alas, in this respect only a record of failure. No sexual cells and no method of reproduction—other than by simple transverse fission—have so far been discovered in any month of the year in which we have found Protohydra alive in the pools.

But in view of the importance of the subject of investigation it has been decided to publish a few notes at once on the natural conditions under which Protohydra was found to be living in this country and the method of its capture, in order that the chances of making the next important contribution to our knowledge of the genus may be shared by others equally with ourselves.

The pools in the tidal marshes of the Hamble river where Protohydra is found are very variable in size but in general of a round shape, a few feet in diameter and not more than four or five inches deep. They are flushed with fresh estuarine water only at spring tides. Between the pools there is a rich vegetation in which during the summer months sea-lavender and spartina grass are predominant features. The water in the pools is usually clear, but is sometimes covered with a bacterial film, and the bottom has a colour like iron-rust due to a continuous thin crust of diatoms. When this crust is disturbed a fine black mud is revealed which has a foul marshy smell, particularly in the summer months. Protohydra lives in this diatom crust, the oral end browsing over the surface for food, the aboral end fixed to the mud just below the crust.

As stated above, when fully expanded, Protohydra does not exceed 3 mm. in length, but the moment it is disturbed it contracts into spherical shape less than ½ mm. in diameter. In consequence of its very small size and of its pale orange colour, which closely resembles the colour of the crust, it is very difficult to observe on the marsh. The only way to be certain whether Protohydra is present or not is to scrape up carefully with a tea-spoon some of the crust, put it into a bottle with marsh-water, allow it to settle in a shallow glass dish for twelve to twenty-four hours, and when a new crust of diatoms has formed in the dish to examine this new crust with a lens.

The bottles containing this mud and water have been sent to Manchester from time to time, and the Protohydra kept under observation in the laboratory without a fresh supply of water for periods of five or six weeks.

A very important feature in the life-history of Protohydra is that in the months of July and August it undergoes some change of habit by which it has entirely evaded our observation. We have never found, notwithstanding most careful search, any trace of Protohydra during these two months.

Greeff found Protohydra at Ostend in September, 1868, in the autumn (Herbst) of 1891 and the spring of 1892. As he failed to find it for twenty years after 1868 and there is no record of his having obtained it in the summer months of any year, his observations in this respect are probably not inconsistent with our own. The disappearance of Protohydra in July may be due to one of three possible causes: it may undergo contraction and encystment or it may produce protected eggs which remain dormant until the autumn, or it may give rise by gemmation or fission to sex-bearing medusæ or floating bodies. If it be due to either of the first two causes the summer stages of Protohydra will undoubtedly be extremely difficult to find in the thick black mud of the pools. To test the possibility of the third cause Mr. Ashby and the writer selected a time just before the onset of the full spring tides towards the end of July this year (1919), and made a careful investigation of the plankton of several pools with a fine tow-net of millers' bunting. We found no medusæ, and, we may add, no evidence of any species of the Cœlenterata in the pools or marshes.

In a problem of this kind there are many methods of attack and we have by no means abandoned the investigation. Still more careful observation of the habits of the animal at different times of the year and a more careful search of the black mud in the summer will be made in the hope of eventually solving the mystery. But the publication of these notes may induce others to search localities with similar conditions to those of the Hamble river and thereby extend our knowledge of the distribution of the species.

A few words may be added here on the constituents of the fauna and flora of the pools. The most abundant of the diatoms that form the thin crust on the mud is Pleurosigma balticum. During the greater part of the year the orange-coloured species of Dinophilus (D. tæniatus) occurred but was especially abundant in the spring. In July Dinophilus entirely disappears as it does elsewhere at that time of year. In the spring we found large numbers of Gromia oviformis and an abundance of ciliate infusoria (including Uronychia sp.? and Actinotricha saltans). Small Harpactid Copepoda seem to be present at all times of the year and so are immature forms of a species of Nematode worm. There is the common shore crab, Carcinus mœnas, in great numbers on the marshes, and in July we found in the pools a late post-larval stage of Palæmonetes varians, and, swimming rapidly on its back like an Apus, the estuarine Isopod, Exosphæroma rugicauda Leach. The writer is indebted to Dr. Tattersall for kindly identifying these species of Crustacea. In the spring we found a few specimens of Paranais litoralis. This species is abundant together with Hemitubifex and Haplobranchus in the pools of the mud-flats at Sheerness, where it was recognised by Sir Ray Lankester in 1887 and described and figured by Sir Alfred Bourne in this Journal, vol. 32, N.S. The only Mollusca we have observed are Paludestrina stagnalis Baxter (i. e. Hydrobia ulvæ Pennant) and a few specimens of an unidentified Nudibranch.

This short summary of the living organisms of the pools in which Protohydra is found is sufficient to indicate that the fauna and flora are typically estuarine in character, and as there is nothing very exceptional or remarkable about them, it seems highly probable that Protohydra will be found in many other estuaries of the British Isles. The best months of the year in which to look for it are probably April and May when it is most actively increasing in numbers by transverse fission. The worst time to look for it is during the months of July and August.

Protohydra is carnivorous but we have not observed the way in which its food is caught. Greeff describes and gives a figure of a specimen that had devoured a Copepod larger than itself. We have also found specimens which had swallowed the Harpactid Copepoda that abound in the water, and we have a preparation of another specimen that had ingested one end of a Nematode worm.

There can be no doubt that the small and microscopic fauna is not so rich in July as it is in the winter and the spring, but as the Harpactids and Nematodes are abundant all the year round and these are the only organisms that have been observed to be the prey of Protohydra, it does not seem probable that the shortage of food is the cause of its disappearance in the summer. The pools do not dry up even at the end of neap tides in the hottest summer months, so that the æstivation is not apparently a provision against drought. The higher temperature of the pools and the increased foulness of the mud may possibly afford the explanation of the phenomenon, but the extraordinary resistance that Protohydra shows to the conditions of living for weeks in a shallow dish in a laboratory without a change of water does not lend this explanation very strong support.

ADDENDUM.—Since the above was written Miss Lebour informs me that Protohydra has also been discovered by Mr. Baker up the Laira river near Plymouth. This discovery confirms our belief that this interesting and probably archaic Cœlenterate is probably a very common form in suitable estuarine conditions.

References.

1. R. Greeff.—'Zeits. f. wiss. Zool.,' xx, 1870, p. 37.

2. C. Chun.—Bronn's 'Klassen u. Ordnungen,' Bd. ii. "Cœlenteraten," 1894, p. 217.

3. W. M. Aders.—'Zool. Anzeig.,' xxvi, 686, 1902, p. 33.

On the Classification of Actiniaria.

Part I.—Forms with Acontia and Forms with a Mesoglœal Sphincter.

By

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With Plate 22 and 32 Text-figures.

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I. INTRODUCTION.

WHILST working out recently the species contained in two collections of Actiniaria (the "Terra Nova" collection (79)[1] and one made by the Irish Fisheries Department (**80**)), I have been led to develop certain views on Actinian classification. Some of the families of Actiniaria are defined by only a small number of the more important characters possessed by the contained forms, with the result that some of them (e. g. Ilyanthidæ, Sagartiidæ, Paractidæ) represent not so much families as series of forms, each series including a number of animals which differ among themselves in ways so important as to be fundamental, although presenting a few common features. This arrangement neither represents the natural affinities of the genera nor does it throw much light upon their evolutionary history. It is often difficult also to allocate new species and genera to their correct position, because in a good many cases sufficiently precise generic definitions have not been arrived at. I have therefore thought it would be the best thing to set out for what they are worth the ideas I have developed during the course of my work. I have as yet applied them in detail to only one particular portion of the Actiniaria, and that application constitutes the following paper. I have begun with the group of forms belonging to the Actiniina which possess acontia or mesoglœal sphincters, because the collections I have been working at happened to present a preponderance of those forms; I am also well acquainted with some living members of it. I am hoping in due course to work out schemes on similar lines for the classification of those Actiniina not included in the present paper and of the Stichodactylina. As yet, however, I have made only a beginning on the two latter groups, so am publishing the finished section at once. More work on these two groups may throw fresh light on various Actinian problems, but is not very likely, I think, to affect the section dealt with in this paper to any extent.

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[1] Numbers in brackets refer to papers in the list of literature on pp. 564–572.

With regard to the principles upon which my classification is based, I am glad to acknowledge my indebtedness to a paper which appeared last year on the question of the systematic position of the Phelliidæ, by Prof. G. C. Bourne (**10**), and which has helped me greatly. I had, however, begun to work out the main ideas involved and to form my families in detail before I knew of Prof. Bourne's paper with its similar ideas.

I should like to record my most hearty thanks to Prof. H. J. Fleure and Prof. G. C. Bourne for help very kindly rendered in different ways, and to Dr. N. Annandale for specimens of some of his Indian anemones.

At the end of the paper I have included a discussion on the relative merits and stability of a number of generic characters, together with new definitions of the genera contained in the group here treated. I have felt this justified by the difficulty I have had in identification and related matters, and have done it in the hope that identification may be rendered simpler by it. In any case it is a convenience to work with a collection of generic definitions, and in the end I hope to make it complete.

In the two reports on anemones already published I have used the ordinary classification almost as it stood because I had not at the time of writing them got far enough with any other scheme. For the purposes of this paper I am accepting provisionally the main division of the Actiniaria into Actiniina and Stichodactylina. As I have mentioned before (**79**, p. 14) I have felt unable to accept Carlgren's division of Actiniaria into Protantheæ and Nynantheæ, and in this I am following McMurrich, Haddon, Duerden and Bourne. The chief difference between these two tribes is that in the Protantheæ there is ectodermal longitudinal musculature in body-wall and actinopharynx, while in the Nynantheæ there is not. Gonactinia and Protanthea are the original Protantheæ. But if with them we are to include all forms possessing this ectodermal musculature, it will mean picking out a form here and there from widely different families and putting them together. The following forms are recorded as possessing the ectodermal musculature, though Carlgren has expressed doubts about it in the case of some of them (the list is not meant to be exhaustive, but merely gives a selection):

Protanthea.
Gonactinia.
Corallimorphus.
Corynactis.
Thaumactis medusioides.
Phymanthus crucifer.
Ricordea florida.
Bunodeopsis spp.
Bolocera brevicornis (but not other Boloceras).
Aiptasia couchi.
Boloceroides.
Actinotryx sancti-thomæ.
Actinoporus elegans.

A more divergent collection could hardly be made. Apart from the ectodermal musculature, their other important characters show that they belong to totally different and unrelated families. The ectodermal musculature of the body-wall is no doubt the survival in a case here and there of a primitive character possessed by the original ancestor of the group and which has been lost in most cases. It is even possible that careful study of the life and habits of the forms possessing it would reveal a reason for its retention in each case. But to use it in classification leads to unnatural results.

There will be found below references to correlation between habit or life conditions and structure. I should like to make a plea for the study of living anemones and their life-habits wherever possible. As Annandale has remarked (**4**, p. 72), it is doubtful whether Gosse was so far from the truth as later systematists supposed when he laid stress upon the study of the living organism in the case of Actinians. At any rate, when one studies living species one seems to gather a much better idea of their relationships and of the meaning of their structure than if anatomy is exclusively relied upon. In the case of allied *species* distinction is sometimes extremely simple when they are alive, but very difficult, if not impossible, when dead, even with the aid of anatomy.

I ought to mention that in this paper I have not founded anything on incomplete descriptions or upon descriptions of doubtful species where that could be avoided. It seems unprofitable to build much upon species of which our knowledge may need revision. I have included in the lists of genera which follow the family definitions at the end of the paper the names of a number of doubtful genera (in a separate list, in each case, from the better-known genera), but this is, of course, quite tentative.

In re-defining the genera I have been unable to avoid a certain amount of re-arrangement of species and splitting or fusing of genera. I have done my best to keep this within the narrowest possible limits, and have pointed out all changes made. Some such revision is bound to take place when all the genera are surveyed together, and it means that the definitions gain in precision and therefore render identification easier, and it has been my aim to express in them the natural relationships of the forms, and to apply similar principles all through, with exceptions in individual cases which seemed to call for special treatment. I may add that I have personal knowledge of living or preserved specimens, and in most cases of sections, of representatives of half the genera defined; in some cases I have added to generic definitions from my own study of the forms in question. My data about other forms are of course obtained from literature. In cases where there are only one or two species in a given genus, it is of course always possible that the addition of new species to the genus may necessitate alteration of minor points in the definition.

Finally, I have not meant the paper to be an exhaustive compilation as regards lists of species and synonymy. I have therefore included, for convenience, just such main synonyms as are strictly necessary. With regard to the lists of species following the genera, these are not in all cases complete; but as far as I know they include at any rate most of the species which have been investigated anatomically, except in one or two large genera.

I wish to acknowledge with thanks the permission to reprint some of the text-figures from other publications. Text-fig. 12 is reprinted from Prof. Bourne's paper on Phelliidæ from New Guinea ('Quart. Journ. Micr. Sci.'), by kind permission of the author. Text-figs. 1, 22, 23 and 32 are reprinted from my "Terra Nova" report, Actiniaria. Text-figs. 24, 25, 27 and 31 are reprinted from my paper on Irish Actiniaria in 'Proc. R. I. Acad.' The other text-figures and the plates are freshly drawn for this paper.

II. Brief Histories of the Sagartiidæ and Paractidæ.

The brief histories which follow do not claim in any way to be complete. Their aim is to simply review the chief modifications undergone by the families since their inception, in such a way as to render the subsequent discussions intelligible. With that end in view I have thought it permissible to repeat a good deal which has been written by various authors, but which is scattered about in different papers.

SAGARTIIDÆ.

P. H. Gosse founded a family which he called "Sagartiadæ" in 1858 (P. p. 415) and defined it thus: "Sagartiadæ. Basis adhærens. Tentacula simplicia, in cyclis continuis digesta. Cutis, pro filis retractilibus armatis emittendis, perforata." The genera included were Actinoloba and Sagartia. In his 'Actinologia Britannica,' p. 9, he gave a fuller English diagnosis, adding nothing essential to the preceding. The genera there included were Actinoloba, Sagartia, Phellia, Adamsia, Gregoria, Discosoma. Sagartia is of course the typical genus of the family, and both Gosse himself (**37**, p. 122) and Haddon later on (**42**, p. 302) have made it perfectly clear that the species regarded by the former, the founder of the genus, as the genotype, was S. miniata. Therefore it will be taken for granted throughout this paper that whatever may happen to the various genera under discussion, the name Sagartiadæ (in its amended form, Sagartiidæ) must be applied to the family containing Sagartia as typified by S. miniata and its immediate relatives.

In 1868 A. E. Verrill ('Proc. Essex Inst.,' vol. v) included the Sagartids as a sub-family under the Actinidæ. He placed Phellia in another sub-family, the Phellinæ, however.

In 1883 A. Andres included Actinoloba, Heliactis, Cylista, Adamsia, Aiptasia, Sagartia and Nemactis in the Sagartidæ, excluding Phellia and Gregoria, placing the former in Phellidæ and not definitely classifying the latter.

In 1882 R. Hertwig endeavoured to establish the family on anatomical grounds. He was, however, unlucky. It so happened that the particular acontiated anemones dealt with in his investigations belonged to a portion of the family in which the members have strong mesoglœal sphincters, and have only six pairs of perfect mesenteries, which are sterile. Even the representative of the genus Sagartia which he had investigated (S. parasitica), happened to be the one species which had been included in that genus by mistake, and which he referred to its correct position in the genus Calliactis. The result of this was that Hertwig made a definition of the Sagartidæ which was based upon the presence of acontia, together with the other anatomical features above mentioned, *but which excluded* Sagartia miniata *and its allies, the true types of the family, from the Sagartidæ.*[1]

In 1889 A. C. Haddon (**42**, pp. 301–305) referred to most of the foregoing facts, and showed that Sagartia miniata and others had more than six pairs of perfect mesenteries, thus differing from the forms described by Hertwig. Haddon considered that the time had not arrived for fully classifying the family, but he constituted as a sub-family a group which he called the Chondractininæ. The definition of this sub-family was fairly similar to Hertwig's definition of the whole Sagartidæ but was narrower, and excluded Adamsia, Metridium and Calliactis as well as Sagartia. It constituted, however, a very natural group within the main

[1] Hertwig identified one of the "Challenger" anemones as Sagartia sp., but, as more recent work has shown, this identification was incorrect. The form in question evidently belongs to the Chondractiniidæ, and is probably a Sagartiomorphe or something similar.

family, and contained the forms best known to its author. The genera included were Chondractinia, Hormathia, Chitonactis, Actinauge and Paraphellia.

In 1892 J. A. Simon recognised two sub-groups within the Sagartidæ—the Aiptasinæ, with the sphincter endodermal or absent, and the Sagartinæ, with a strong mesoglœal sphincter. Among the latter he distinguished Sagartians without, and Phellians with, a cuticle.

In 1893 J. P. McMurrich adopted Haddon's Chondractininæ, at the same time suggesting that it might be almost identical with Verrill's Phellinæ. However, the anatomy of Phellia was not then understood. McMurrich had previously suggested himself a division of the Sagartidæ into Phellinæ and Sargartinæ, but he adopted Haddon's subfamily as being more extensive than, and probably including, the genus Phellia. His arrangement therefore is—

Sub-family Sagartinæ.

"Sagartidæ with the ectoderm naked, the acontia being emitted from the mouth and through the column-wall, in which definite openings (cinclides) are present (always ?) for their emission."

Sub-family Chondractininæ, Haddon.

"Sagartidæ with thick column wall, usually with the upper portion (capitulum) different in character from the lower (scapus) and capable of being entirely invected: the scapus provided with an external cuticle and usually nodulated or warty; the sphincter strong and embedded in the mesoglœa; only the six primary pairs of mesenteries perfect and at the same time nongonophoric; acontia emitted by the mouth only, there being no cinclides."

This definition of the Chondractininæ is not exactly Haddon's original one, but does not differ from it in anything important.

Also in 1893 O. Carlgren (**12**, pp. 86–7) discussed the matter. He showed that in some Sagartias the primary mesenteries were *fertile*, thus still further removing them from Hertwig's Sagartidæ. He kept to the Sagartinæ and Phellinæ, but added a new sub-family, the Metridinæ. His Phellinæ was practically identical with Haddon's Chondractininæ, but he included Phellia in it and went back to the old name; Phellia was still not understood as regards its anatomy, although Verrill had said a little about it in 1867.

It will be noted that by this time the "Sagartidæ" exhibited only one differential character common to all of them—the presence of acontia. Aiptasia had proved an exception to the mesoglœal sphincter rule, the number of perfect mesenteries and distribution of gonads were shown to be variable, and cinclides were proved to be present in some cases and absent in others. Cuticle also was present or not in different cases. Even a pedal disc was not invariably present (e. g. Ilyactis).

In 1897 C. R. Kwietniewski (**55**) discussed the family at length, and noticed that the acontia present practically the only feature common to all members of it. He ends up with a division of the family similar to Simon's.

In 1898 A. C. Haddon (**44**, pp. 446–7) briefly reviewed the family history and adopted a new arrangement of sub-families—an arrangement also suggested in 1898 by O. Carlgren. By this time more was known of the anatomy of Phellia, and it was realised that it was quite different from the Chondractininæ, and must be placed in a sub-family apart, which sub-family, of course, was Verrill's original Phellinæ, containing Phellia only. I repeat Haddon's definitions here in full, since they represent the most recent classification of the family before 1918, and are important for reference.

Family SAGARTIIDÆ, Gosse.

"Actiniinæ with a contractile pedal disc; body-wall smooth, or provided with verrucæ or tubercles, and usually perforated by cinclides, with or without a cuticle. Tentacles usually numerous and retractile, not very long, smooth, simple and generally entacmœous. Sphincter muscle characteristically well developed and mesoglœal, occasionally diffuse endodermal, or even absent. At least six pairs of perfect mesenteries; the first cycle of six pairs of mesenteries may be fertile or sterile. Acontia present."

Sub-family AIPTASIINÆ, Simon.

"Sagartiidæ in which the sphincter muscle is either absent or very feebly developed and mesoglœal, or diffuse and endodermal."

Sub-family Sagartiinæ, Verrill.

"Sagartiidæ with more than six pairs of perfect mesenteries, of which the six pairs of primary mesenteries are fertile, except the directives in some species; body-wall soft; no tubercles, but verrucæ (suckers) may be present in the upper part of the column; cinclides present (chiefly ectodermal invaginations); one or two gonidial grooves; strong mesoglœal sphincter muscle."

Sub-family Phelliinæ, Verrill.

"Sagartiidæ with usually an elongated column, the capitular portion of which is generally delicate and extensile; body-wall provided with a cuticle, but without any solid or hollow processes, such as tubercles, vesicles, or suckers; no cinclides. Tentacles simple, neither very numerous nor very long. Only six pairs of perfect mesenteries which alone are fertile. The remaining mesenteries are usually feebly developed. The retractor muscles are very strongly developed on the primary mesenteries. Acontia usually feebly developed, and emitted only through the mouth. Strong mesoglœal sphincter muscle."

Sub-family Metridiinæ, Carlgren.

"Sagartiidæ with six or more pairs of perfect mesenteries, of which the six pairs of primary mesenteries are sterile; usually one gonidial groove with its pair of directives, but more may occur; body-wall relatively thin, and without a cuticle; cinclides present (chiefly endodermal evaginations); well-developed mesoglœal sphincter muscle."

Sub-family Chondractiniinæ, Haddon.

"Sagartiidæ with only six pairs of perfect mesenteries, which alone of the well-developed mesenteries are sterile; two gonidial grooves and two pairs of directives; body-wall usually thick, with a cuticle and often nodulated; cinclides absent (?); acontia rarely emitted, and then by the mouth only; strong mesoglœal sphincter muscle."

Of these sub-families, the Sagartiinæ corresponds to the Sagartinæ used by Carlgren in 1893; the Metridiinæ to Carlgren's sub-family with Aiptasia removed; and the Chrondractiniinæ to Carlgren's Phelliinæ with Phellia removed from it.

In work published more recently (1898, 1904), J. P. McMurrich did not accept Haddon's arrangement for various reasons, and returned to Carlgren's three sub-families.

In 1918 G. C. Bourne (**10**) published descriptions of a number of Phellids, and discussed very fully the relationships of the Pheliinæ (as understood by Haddon) to the other Actiniaria. He limited the Phelliinæ to Phellia, Decaphellia, and possibly Halcampactis, agreeing with Haddon in separating these forms from the Chondractiniinæ. He analysed the characteristics of the Phelliinæ, discussed the occurrence of certain of their characters, considered singly, among other Actiniina, and showed quite conclusively that, going by *the sum of the more important characters* rather than by one or two somewhat arbitrarily selected, they incline on the balance rather to the Halcampidæ than to any of the sub-families of the Sagartiidæ. He showed that acontia, the mesoglœal sphincter and certain other characters vary independently within the Actiniina. He adopted a hypothesis which would account for the appearance or disappearance of acontia in groups having very different combinations of other characters, and claimed that this would get rid, once for all, of the idea that the presence of acontia is such a positive mark of inter-relation that all forms possessing it must be united in a single family. The main conclusions reached were that the Sagartiidæ must be broken up, that the Phelliinæ must form a distinct family approximating to the Halcampidæ, and that the sum-of-the-characters principle must be generally applied in classifying the Actiniina.

PARACTIDÆ.

R. Hertwig founded a family which he called Paractidæ in 1882 (**51**, p. 41), and which he defined as "Hexactiniæ, with numerous perfect septa, and with very contractile, moderately long tentacles, which can be completely covered; circular muscle very strong, mesodermal." He placed in this family, as his first genus, Paractis, M. Edw., and also included Dysactis, Tealidium, Antholoba and Ophiodiscus.

In 1883 A. Andres (**2**, p. 471), set up, independently of Hertwig, a sub-family Paractidæ of his family Actininæ. He defined it thus:

"Forma.—Base più o meno aderente. Colonna variiforme, contrattile, liscia, senza pori, tubercoli, verruche o rivestimenti. Margine talora presente ma semplice e non sviluppato; non mai crenulato e fornito di acroragi. Tentacoli policicli, conici; retrattili; Colore, Dimensione e Giacitura varii." This family contained the genera Paranthus, Paractinia and Paractis.

Of these two families that of Hertwig has priority. The exact standing of the forms referred by Andres to his own family is not yet known in all cases, but Paranthus has been anatomically investigated, and has been referred to Hertwig's Paractidæ on structural grounds. It is hardly probable that Andres' family will stand, even under a new name, when the anatomy of all its members is known. Its forms can be referred to their correct positions one at a time as more is known of them. It is the family of Hertwig, to which the name Paractidæ rightly belongs, that we shall follow here.

In 1893 J. P. McMurrich (**60**, p. 160) widened the definition so as to include forms with few perfect mesenteries, and he omitted any definition of the tentacles.

In 1893 O. Carlgren (**12**, p. 64) gave a history and discussion of the family. He re-defined the family as follows: "Actininen mit Fussscheibe, mit sehr contractilen, mässig langen Tentakeln und zahlreichen, vollständigen Septen. Septenpaare der höheren Cyclus (vom dritten oder vierten an) unregelmässig entwickelt, so dass das Septum, das seine Längsmuskeln gegen den im Allgemeinen nächst niederen Septencyclus kehrt, mehr entwickelt ist als das andere in demselben Paare. Radialmuskulatur der Mundscheibe und Längsmuskulatur der Tentakeln im Allgemeinen mesodermal. Sphinkter mesodermal. Acontien und Cinclides fehlen." But in a note at the end of this paper (**12**, p. 137), in the light of some new information regarding the genus Paractis, he transferred the above definition from the Paractidæ to a new family Actinostolidæ, containing the genera Actinostola and Stomphia, and made a fresh diagnosis for the Paractidæ proper, including Paractis. This diagnosis was "Actininen mit Fussscheibe, mit mässig langen Tentakeln und gewöhnlich zahlreichen, vollständigen Septen. Septen in demselben Paare regelmässig entwickelt. Radialmuskulatur der Mundscheibe und Längsmuskulatur der Tentakeln im Allgemeinen mesodermal. Sphinkter mesodermal, gewöhnlich wohl entwickelt. Acontien und Cinclides fehlen."

There is little to add. Hertwig's Paractidæ has been generally accepted with modifications, and the Actinostolidæ and Paractidæ as defined by Carlgren in 1893 have become sub-families—Actinostolinæ and Paractinæ—within the main family (see Carlgren, 1899, pp. 26-7). The features possessed in common by the forms which have been included in the family from time to time are the presence of a base and of a mesoglœal sphincter and the absence of acontia and cinclides.

In 1918 O. Carlgren (**23**) contributed fresh details of the anatomy of Polysiphonia tuberosa Hertwig, and claimed that on account of the very unusual way in which its mesenteries develop it should be placed in another sub-family (Polysiphoniinæ) from the Paractinæ and Actinostolinæ, or in a family Polysiphoniidæ if it should be considered that the Paractinæ and Actinostolinæ had more than sub-family rank. Details of the Polysiphoniinæ will be found on p. 549.

III. Description of Certain Anatomical Details.

I should like to establish one or two anatomical particulars before going further, because some of the statements which will be made later on are based upon them.

Aiptasia Couchii, Cocks.

It has long been impossible to define the genus Aiptasia quite exactly, because of the absence of any description of the anatomy of British specimens of the genotype—A. couchii. Pax has described, it is true, the anatomy of an Aiptasia from Gomera in the Canary Islands, which he identifies as A. couchii (see **70**, p. 337); but since it does not agree in all respects with the structure I find to be characteristic of actual British specimens, I conclude that it may be a different species closely allied to A. couchii. One might easily expect this to be the case in a locality so far from Britain as Gomera. I have obtained from Plymouth several living specimens of A. couchii, one of them large and the others rather small. These agree completely with Gosse's description of the species. A study of sections of some of them reveals the following details. Six pairs of mesenteries alone are perfect, including the two pairs of directives. There is no sharp distinction between the primary mesenteries and those of the other cycles, save as regards the perfection of the former only—i.e. the mesenteries are not distinguished into macrocnemes and microcnemes.[1] The retractor muscles are fairly weak and perfectly diffuse (see Text-fig. 20). My material unfortunately does not show which mesenteries are fertile. Well-developed acontia are present and the mesenterial filaments possess ciliated lobes. Cinclides present. Longitudinal musculature of tentacles and radial musculature of oral disc ectodermal. At the extreme margin of the body is a very weak sphincter muscle, completely embedded in the mesoglœa. It is quite poorly developed, and consists, as seen in section, of a thin chain of cavities in which and round the borders of which the muscle-fibres are arranged. My sections of it are very clear, and admit no possibility of doubt as to its existence. A small portion of this sphincter is shown in Pl. 22, fig. 10, which also exhibits the large zooxanthellæ with which the endoderm is crowded. In some sections it is possible to see quite clearly that in the uppermost parts, at all events, of the body-wall and actinopharynx an *ectodermal* longitudinal musculature is present. It is feebly developed, but in some cases the fibres are supported by distinct processes of the mesoglœa. I have drawn a small portion of a transverse section of one of the actinopharyngeal grooves in Pl. 22, fig. 11, where these short processes seem best developed. Apparently a nerve-layer accompanies this musculature.

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[1] See p. 456 for an exact definition of the terms "macrocneme" and "microcneme."

The Aiptasia from Gomera described by Pax as A. couchii differs from the true British species in that it possesses no mesoglœal sphincter. Also, it can have more tentacles—72, whereas our form seems limited to 48. There are certain anemones in which there is apparently a constant maximum number of tentacles. Further, Pax does not mention that his anemone has any ectodermal musculature in body-wall and actinopharynx. These differences are enough to separate it from A. couchii, and I propose for it the name A. paxi. Its generic name should be Aiptasiomorpha (see p. 530), not Aiptasia. The effect of the discovery of a mesoglœal sphincter in A. couchii on the generic synonymy is discussed on p. 531.

SAGARTIA MINIATA, Gosse.

This is the type-species of the genus Sagartia, and a short statement of its essential characteristics will help to make later discussions clearer.

The body-wall is smooth when distended, finely corrugated otherwise, and is provided with more or less conspicuous suckers which are at their best development on the upper part of the body. Cinclides are present, but I will leave the more detailed description of these till the next section (see p. 451). Tentacles typically in five cycles, on the plan $12 + 12 + 24 + 48 + 96 = 192$. This formula is liable to variations affecting one or more sectors of the animal. The lips are strongly corrugated. The species is clearly characterised and separated from its nearest relatives by colour and markings, but that does not especially concern us here and I hope to deal with it in another place. A fairly large and quite typical individual of which I have a series of sections has five cycles of mesenteries. They are developed with perfect regularity save in one sector, in which two tertiary pairs are absent, their absence carrying with it the further absence of two pairs belonging to the fourth cycle, and four belonging to the fifth. This is simply a small individual irregularity, hardly any specimen of this species developing exactly according to formula. The mesenterial formula for this specimen, then, is 6 p. + 6 p. + 10 p. + 22 p. + 44 p. = 88 p. Of these, two pairs are directives and are related to the two actinopharyngeal grooves. Twenty-two pairs are perfect—cycles 1–3 inclusive. Only the perfect mesenteries have definite retractor muscles, and these are strong and diffuse, with slender well-branched processes (see Text-figs. 13–16). There is no sharp distinction of the mesenteries into macrocnemes and microcnemes,[1] the cycles being graded. Every perfect mesentery bears two mesenterial stomata—a small labial and a larger parietal. The zone containing the labial stomata is about 1·7 mm. deep, and that containing the others about 2·6 mm. Filaments occur on mesenteries of cycles 1–4 (inclusive); they are well developed. Ciliated lobes are present. The mesenteries of the fifth cycle are very small, and are better developed in the highest and lowest regions of the body than elsewhere. Sections and dissections of other specimens show that gonads develop on mesenteries of cycles 1–3 inclusive, at least, even the directives being fertile in some cases at any rate. Acontia are present in abundance. Definite basilar muscles are developed. The longitudinal musculature of the tentacles and radial musculature of the oral disc (see Pl. 22, fig. 9) are ectodermal. There is a strong and definite mesoglœal sphincter, and I have drawn a typical section of it in Pl. 22, fig. 3.

HALCAMPA CHRYSANTHELLUM, GOSSE.

I have discussed the synonymy of the genus Halcampa in a former paper (**79**, pp. 8–10) in correlation with the possession of a mesoglœal sphincter by the genotype, H. chrysanthellum. I am now able to give a figure showing a small portion of a section of this sphincter in detail (Pl. 22, fig. 7). Its general situation in the body-margin is better understood from Text-fig. 1.

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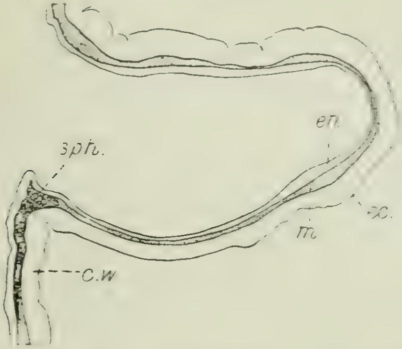
[1] See p. 456 for definition of terms "macrocneme" and "microcneme."

The specimen illustrated is from Plymouth. The sphincter in this species is small and perhaps weak, but distinct.

TEXT-FIG. 1.



Longitudinal section of a tentacle and of the body-margin of **Halcampa chrysanthellum** to show position of sphincter. *c.w.* Column wall. *ec.* Ectoderm. *en.* Endoderm. *m.* Mesoglœa. *sph.* Sphincter.

FLOSMARIS PHELLIOIDES, n. gen., n. sp.

The single specimen of this species which I have been able to examine was kindly lent me by Prof. J. Stanley Gardiner. It is labelled "Hulule: sand—close to low tide."

There is a quite distinct pedal disc, which is smooth and very thin-walled and flabby, showing the mesenterial insertions through its wall. The column is divided distinctly into scapus and capitulum; it is longer than wide, and the scapus is of much greater extent than the capitulum. Wall of scapus fairly thin throughout, not stiff enough to support itself; in the lower part excessively thin, flabby, and semi-transparent. This thinnest lower part is smooth and devoid of sand-grains; but the main part of the scapus is more opaque, though not entirely so, and is covered with many small papillæ or suckers to which white grains of sand are firmly attached. The capitulum is fairly smooth and devoid of sand-grains. Margin tentaculate. I could see no trace of cuticle even on the scapus. Whether cinclides are present or not is uncertain, but careful examination of the wall failed to reveal any, and I think they are probably absent.

Internally there are twenty-four pairs of mesenteries visible to dissection. These are sharply divided into macrocnemes[1] and microcnemes. The first two cycles (6p. + 6p.) are macrocnemes; every one of them possesses a circumscribed retractor plainly visibly to the unaided eye, a large gonad, and a well-developed filament; all of them are perfect. There are two pairs of directives. The microcnemes form simply twelve pairs of narrow streaks on the body-wall, and in the portion of the animal dissected they bore no trace of gonad, filament, or retractor, so that if any of these were present they would be so small as to be invisible to the naked eye or to a lens. These microcnemes are of course not perfect. Well-marked but not greatly developed acontia are present on some at least of the macrocnemes. In the case of the macrocnemes there is a tendency for the gonad and filament to be more extensive, or to end at a lower level in the cœlenteron, on one partner than on the other, of each pair; the latter point affects the retractors too. In the case of the primary macrocnemes, the eight Edwardsia-mesenteries have the endings of these structures rather lower than the other four primaries; and the four lateral mesenteries of these eight have them ending lowest of all. The lowest ends of those of the secondary macrocnemes are generally speaking rather higher up than those of the primary ones, and here, if those of one partner end lower than those of the other, it is the ventral and not the dorsal partner in each pair which is affected—the opposite of what takes place in the case of the primaries. I should add that the above statements are made from a very careful dissection of one-half of the lower three-fourths of the animal (the uppermost end being devoted to sections), aided by a less detailed study of the other half

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[1] See definition on p. 456.

—I did not wish to break the specimen up too much. But the animal is large and well preserved, and what I did see of the second half without injuring it unduly allowed me to see that in most respects it is certainly, and in the rest probably, exactly identical in essentials with the first. It will be noted that although there are two cycles of macrocnemes here, the transition from cycle 2 to the microcnemes is very abrupt, there being no intervening cycle or cycles of moderately developed mesenteries between the fully-developed macrocnemes of cycle 2 and the rudimentary microcnemes of cycle 3.

Sections of the upper part of the body confirm many of the above details and provide others. The retractors of the macrocnemes vary in size in individual mesenteries, but as a whole there is not much difference between the primary and secondary macrocnemes in this respect. The macrocnemes and microcnemes bear well-developed parietal muscles. In the uppermost part of the body some additional microcnemes, over and above the twelve pairs visible on dissection, are present. Some of the microcnemes bear acontia at a fairly high level. The longitudinal muscle of the tentacles and radial muscle of the disc are fairly developed and ectodermal. A mesoglœal sphincter is present, but it is rather small and appears to be entirely confined to the upper part of the capitulum, just below the margin. I cannot as yet be sure about cuticle, but although there is evidently no conspicuous development of it, a certain amount may perhaps be present on the scapus. I hope to add to this description sooner or later.

IV. Discussion of Acontia and Cinclides.

(In relation to the scheme of classification which follows.)

Acontia.

It will be evident from the foregoing brief account of the history of the family Sagartiidæ that the majority of authors consider the chief diagnostic character of the family to be the possession by its members of acontia. Under the heading "Sagartiidæ" has been gathered together a miscellaneous collection of forms differing very considerably among themselves, but possessing no other common feature save the acontia. There is, it is true, a second character found in the majority of them—a mesoglœal sphincter—but this is not universal and occurs in many forms devoid of acontia.

One might say that all anemones are related to one another because all have mesenterial filaments. Although this is the case, it in no way follows that all anemones must be included in one family, or considered as being very *closely* related, on account of that single feature, however much they may differ among themselves in other ways. Now an acontium can only be regarded as a specialised form of mesenterial filament. It is distinguishable from the latter by certain histological details and by the fact that it is free from the edge of the mesentery save at one end. There is therefore no reason why two given forms should be placed in the same family or even considered to be very closely related because both possess acontia if they are very different in other ways. It is quite a possibility, as Bourne has pointed out (**10**, p. 81), that acontia (which are not all identical in structure) may have been independently acquired by several groups of Actinians. I am personally of the opinion that it is more probable that all forms possessing them are descended from a common ancestor, as I shall repeat with what evidence there is in favour of my view later on. But even if this is the case, we do not know how old the group is, and it may be quite old enough to allow for a good deal of divergent evolution since the acquisition of acontia. There is no reason to suppose that that acquisition may not have been at a fairly early stage, since an acontium is but a variety of filament, and filaments are present even in the earliest known anemones. This does not, however, dispense with the acontium character as a useful one in classification, provided it is used as one among others and is not made more important than anything else.

A transverse section of a typical acontium is shown in Pl. 22, fig. 4. The figure displays the general kidney-shaped form typical of acontia in section, the axis of mesoglœa, the large thick-walled nematocysts arranged fanwise on one side of the acontium, and the presence of granular gland-cells. This particular acontium is one belonging to Sagartia miniata, and is stained with borax carmine and picro-indigo-carmine, which picks out the sting-cells very clearly.

Acontia, in general, are slender white or coloured threads attached to the borders of mesenteries at one end and quite free at the other. Their surface is ciliated, and if a piece of acontium is detached from the animal and placed on a slide in a little sea-water, it may exhibit fairly active serpentine movements on its own account for some time. An acontium is mainly characterised histologically by the possession of numerous and often extremely large thick-walled nematocysts. It has also a delicate layer of longitudinally disposed muscle-fibres, which do not always bear the same relation to the axis of mesoglœa. Many gland-cells may be present. A simple mesenterial filament resembles an acontium in general form and in the possession of gland-cells and nematocysts, but it lacks muscle-fibres and its nematocysts are typically smaller and less numerous.

Acontia are emitted more or less copiously through the mouth (according to the kind of anemone possessing them) under certain circumstances, such as irritation. The freedom with which they are put forth varies in different species. They doubtless help to paralyse prey and to defend the animal. If there is a wound they come out through it, and if pores are present in the body-wall these also may be used as means of egress. The cilia covering the acontia may help in their extrusion, but the chief facts about their emission will be dealt with in considering the cinclides. Acontia are more abundantly and better developed in some genera than in others, and in some cases may be partially reduced or even quite rudimentary, so that great care is needed to detect them.

Cinclides.

The definite pores which occur in the body-walls of certain anemones, and to which Gosse gave the name *cinclides*, seem to have been first noticed by Rapp in Calliactis parasitica ("Sagartia" parasitica). Various authors have confirmed the fact of their existence. Gosse considered that the purpose of the cinclides was to let out acontia, and a connection between the two often seems to be assumed. It is an idea which has gradually gained weight in my mind, that the relationship between acontia and cinclides is really secondary and in a sense accidental, and that the acontia emerge through the cinclides *because the cinclides happen to be there.* If this is the case, what is the primary function of the cinclides? It seems likely that they are structures typical of more or less delicate-bodied[1] anemones which provide for the establishment of a relationship between the water in the animal's cœlenteron and the sea outside; and that they provide a line of least resistance for the escape of some of this water on sudden violent contraction of the animal, thus preventing a definite rupture of the wall. There is a good deal of evidence in support of this view, which I will set forth.

In the first place, a number of anemones possess cinclides but have no acontia. The pores found in these species have not before been actually termed "cinclides" as a rule, but in structure and relationships they are identical with some Sagartian cinclides, and I shall henceforward apply the name to them. I had the opportunity of observing, through the kindness of Mr. H. C. Chadwick, of the Port Erin Biological Station, the habits of two living specimens of Peachia hastata in good health. This species has no acontia, but its physa is perforated by very many cinclides arranged in longitudinal rows. It is not easy to observe these cinclides in a living animal under water, because it does not keep still, and is ever changing its shape. However, I succeeded in getting a clear view of them, even with a one-sixth objective. They have the appearance of little windows or thin diaphragms in the body-wall, which are pale by transmitted light; and in the centre of the window is a small aperture the size of which varies from time to time.[1] The animal, therefore, keeps these pores open, sometimes at any rate, when at rest and in a healthy condition, and it seems evident that they have some connection with water-currents. In longitudinal sections of the cinclis-region of one of these specimens one can see that the pores may have a structure similar to some of the cinclides in Sagartia. In some cases it seems that they originated by an ingrowth of the ectoderm, which pushed its way through into the interior. One such *ectodermal invagination* is figured in Pl. 22, fig. 6. It will be seen that the mesoglœa and muscular layer are interrupted by the penetrating ectoderm, which is itself penetrated by a definite channel. The resemblance of this cinclis to the one drawn in Pl. 22, fig. 2, from near the edge of the base of Sagartia miniata is most striking.

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[1] The wall of the body in the genus Calliactis may be fairly thick and firm, and is therefore less delicate than in many cinclis-bearing forms. It is perhaps a case in which strength of body-wall will eventually be gained at the expense of loss of the cinclides.

Other anemones possessing cinclides but no acontia are the following: Hertwig describes pores in the physa of Halcampoides clavus which, from both his description and figures, are evidently ectodermal invaginations comparable to some of those near the base in Sagartia miniata. Prof. G. C. Bourne tells me that he has found pores in the physa of an Edwardsia, whose relations are just those of cinclides. Harenactis attenuata has a definite longitudinal row of cinclides communicating with each exocoel and each endocoel in the upper part of the long vermiform body. Other instances might be mentioned.

Let us now turn to those forms in which both acontia and cinclides occur. Gosse discusses cinclides in his 'Actinologia Britannica,' pp. xxv–xxix. He records observations of *open* cinclides in specimens which were presumably *at rest;* and that a very thin membrane may often be detected across the mouth of an open cinclis. He attributes this membrane to the constant sloughing of mucus which takes place in anemones. He records also that in an animal which had emitted acontia there were open cinclides through which no acontia protruded as well. He attributes the emission of the acontia to the currents of water which are forced out of the animal when it contracts, and notes in connection with this that acontia frequently come out of cinclides as *loops*—not free end first—and that more than one acontium may protrude through one cinclis. G. Y. Dixon (**27**) subscribes to Gosse's account of the cinclides from his own observations, except as regards the thin film across the mouth of an open cinclis. Dixon states that in fully expanded animals (he is dealing with Sagartia venusta, S. nivea, and S. miniata) when at rest the cinclides are generally open, and suggests that the emission of acontia is not their only function, but that, as Agassiz has supposed, they take part in providing communication between the interior of the animal and the surrounding water. Milne-Edwards attributes the liberation of water as well as acontia to the cinclides, and Hertwig suggests the passing in and out of water as the function of the pores of Halcampoides clavus.

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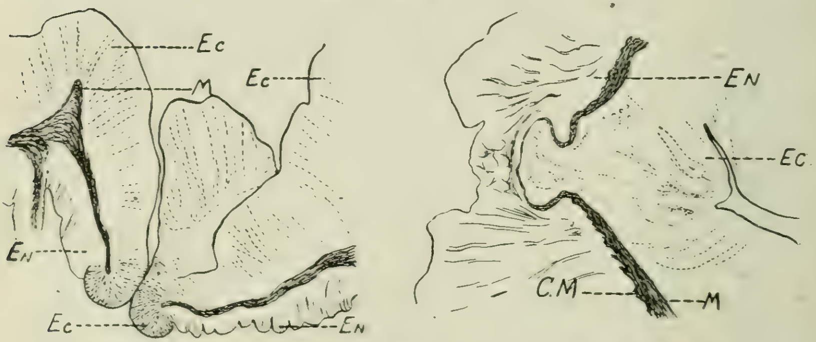
[1] I am not sure that the "window" is invariably perforated, but it is at least frequently so.

I have not experimented at all exhaustively with anemones with regard to cinclides, but what observations I have made are enough to show that the connection between acontia and cinclides is secondary and accidental. If one suddenly squirts a little water containing carmine powder (I did it with S. miniata and C. pedunculatus) into an anemone's mouth with a fountain-pen filler, when the anemone is expanded and pretty well distended with water, this causes the body to contract suddenly and violently, and one can then see very clearly that little jets of carmine are projected with great force through the cinclides, and travel to a considerable distance from the animal before being lost in the water. They come out *instantaneously*, the cinclides acting as safety-valves against rupture of the wall at the first contraction-jerk, the main volume of the carmine returning more slowly through the mouth. These jets of water and carmine are *not* generally followed by the protrusion of acontia quite immediately, the latter seeming on the whole to be forced out only after a good deal of contraction. And of course in any case the mouth is their chief exit. Apart from this I have seen, under the microscope, water spurting from the cinclides of a contracting animal. On one occasion I had a Sagartia miniata under the microscope, and on gently pressing it I saw a jet of water come out of a cinclis, followed by an acontium. I have also seen the acontia forced out by the water as loops, forced into the tentacles by water currents, and forced through the base of an animal just removed from a stone. In the last case the water would be expelled from the contracting animal through small invisible wounds or very thin places such as do occur in the pedal disc, and acontia would accompany the water.

All this seems to show that the acontia come out anywhere where the least resistance is offered, that the cinclides act as safety-valves for water, and that the acontia come out of them incidentally, being propelled in all directions by the escaping water. What, if any, other functions the cinclides have I do not know, and a good deal of investigation, not necessary for my present purpose, would be needed to find out.

In Sagartia miniata and Cereus pedunculatus they sometimes seem to close when one would expect them to be open. In those cases in which I have most clearly observed open cinclides in one of these species when completely *at rest*, there was certainly a film such as Gosse described stretching across the open mouth of the cinclis. This film may be the *diaphragm*, which will presently be described as characteristic of certain cinclides, and which, as is shown by sections, may or may not be penetrated by a definite channel or aperture. It would be quite natural for the aperture, if present, to be kept closed by its sphincter in a state of rest—some of the time at any rate. When a cinclis emitted water, either the hole in the diaphragm would be opened (this I have seen taking place), or else the water would rupture the diaphragm in its excessively thin central part, which, as will be seen, is specially constructed so as to heal again easily. In Peachia the cinclides were in many cases, when I saw them, quite definitely open—the aperture in the centre of each " window " being quite clear and free from a covering film. In this species the life-conditions are not those of Sagartia, and it may well be that the cinclides have a more extended function connected with the frequent change in form of the body as well as that of acting as safety-valves.

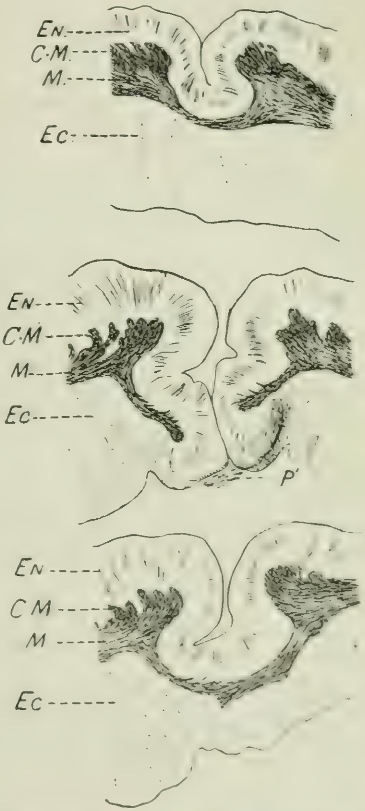
TEXT-FIGS. 2 AND 3.



Sections of cinclides of Sagartia miniata. Fig. 2 on left, 3 on right. See p. 452. *C.M.* Endodermal circular musculature. *Ec.* Ectoderm. *En.* Endoderm. *M.* Mesoglœa.

It seems, therefore, a fair conclusion from the abovementioned data that cinclides and acontia are characters which may have been independently acquired, and have become more or less secondarily connected in some cases. It does not seem unlikely that the cinclides came first, as those species which possess them but do not possess acontia are fairly primitive forms. This being so, although there is no reason why " presence of acontia " and " presence of cinclides " should not be used as two among other characters in classification, we are not necessarily to suppose, when the two are found in conjunction, that this forms a high double specialisation, all forms possessing which must be united in a single family. It should also be borne in mind that many forms possessing acontia are devoid of cinclides, and this makes it an additional advantage to treat the two characters separately.

TEXT-FIGS. 4–6.



Sections of cinclides of Sagartia miniata. Fig. 4 above, 5 in centre, 6 below. Lettering as on Text-fig. 2, p. 450. Also P^1. Disintegrating epithelial mass.

Before leaving the question of cinclides, I think it will be of value to describe their mode of occurrence in a single typical case. Sagarta miniata affords a convenient example. In a specimen of the species of which I have a complete series of sections the following details are found. The uppermost margin of the body is occupied by the sphincter muscle, so that a zone of about 2·3 mm. below the margin is quite free from cinclides, which would interfere with the sphincter if they penetrated it. Below this is a zone about 2·8 mm. deep in which occur many cinclides. This is followed by a wider belt about 3·4 mm. in extent, which is devoid of cinclides. Lastly, the zone of the body-wall immediately above the base, a zone about 1·3 mm. deep, is again occupied by cinclides. That is to say, there are two zones in which cinclides occur, separated by a barren zone. The lower zone is as near the edge of the pedal disc as possible, and the upper zone is as near to the uppermost part of the body as it can be without interfering with the sphincter. This distribution is significant in connection with the function of the cinclides as safety-valves, etc., because it means that they occur near the corners of the exocœls and endocœls which are furthest from the mouth or main exit. Furthermore, the zone in which the upper cinclides occur coincides exactly with that occupied by the labial and parietal stomata of the mesenteries, so that the pores allowing water to pass through the mesenteries are on a level with those allowing it to escape through a wall.

It is evident that the cinclides of the lower zone are not typically identical in structure with those of the upper. One may say that frequently the upper-zone cinclides are chiefly or wholly outpushings or *evaginations* of the *endoderm*, whereas those of the lower zone are often *invaginations* of the *ectoderm*. Moreover, a more complex structure may be detected in many of the upper cinclides than I have seen in any of the lower ones examined. Text fig. 2[1] and Pl. 22, fig. 2, show two good examples of lower-zone cinclides. Both show an interruption in the rather thin mesoglœa (*M.*), and a channel penetrating the ectoderm (*E.*, *Ec.*), which has pushed its way right through the wall and forms two little lips on the inner side. Text-fig. 3 shows another, in which the ectoderm is just in the act of penetrating the mesoglœa, but has not actually gone through the endoderm yet. Text-fig. 4, on the other hand, shows one of the upper cinclides in a young condition, in which it is growing out *through* the mesoglœa *from* the endoderm. Text-fig. 6 is a section cut a little to one side of the centre of the same cinclis as that shown in Text-fig. 5, and it demonstrates the outpushing from the endoderm very well. Text-fig. 9 shows another similar case, the section here also being cut a little to one side of the central perforation. Text-figs. 5, 8, and Pl. 22, fig. 1, show sections passing exactly through the centre of three of these upper cinclides. In these cases it will be seen that the main mesoglœa and the endodermal circular muscle cease abruptly on either side of the

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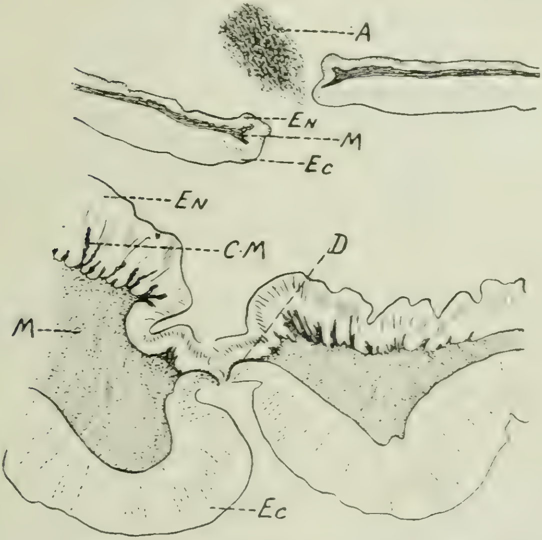
[1] In all the text-figures of cinclides (2–9) I have indicated the general directions of the endoderm-cells by continuous strokes, those of the ectoderm cells by interrupted lines. The mesoglœa is dotted or shaded more darkly.

TEXT-FIGS. 7 AND 8.



Sections of cinclides of Sagartia miniata. Fig. 7 above, 8 below. Lettering as on Text-fig. 2, p. 450. Also *A*. Acontium. *D*. Diaphragm.

cinclis, but that stretching across the interrupted part there is a little very delicate diaphragm (*D.*), consisting of practically nothing but muscle-fibres. The diaphragm itself is interrupted in its middle, putting the ectoderm and endoderm in communication with one another. But although there is also sometimes a definite channel penetrating the epithelium as well as the diaphragm (Text-fig. 5), this often seems to be absent or at any rate irregular (Text-fig. 8, and Pl. 22, fig. 1[1]). (In preserved specimens, of course, the body-wall is often in a somewhat contracted condition, and the cinclides seen in sections are generally closed ones, the two opposing walls of the channel, when it is present, being pressed against each other.) In some cases one can see that in *both longitudinal and transverse* sections the muscle-fibres composing the little diaphragm are cut *crosswise.* This implies that they run circularly round the diaphragm and form a little sphincter-muscle, which in life is no doubt capable of keeping the orifice of the diaphragm closed. In Pl. 22, fig. 5, is seen the diaphragm of a cinclis, much enlarged, as seen with a $\frac{1}{12}$ oil-immersion objective; it shows the muscle-fibres (which must be derived from the endodermal circular muscle) cut *crosswise* in a *transverse* section of the whole body. Longitudinal sections, if clear, present a similar appearance. Text-fig. 7 shows an open cinclis with an acontium partly protruding through it.

The fact that some of the cinclides show a definite interruption in the thin muscular diaphragm, but not an actual passage through the epithelium, seems to give additional confirmation to the idea that they act as safety-valves. It is possible that the cinclides of this type, when first formed, are not exactly pores, but are organised "thin places," or "soft spots," or "lines of least resistance," through which water will burst if through any part on sudden violent contraction of the body. It is noticeable that the parts which in these cases present a definite interruption (the muscle and the mesoglœa) are those which would be least easily renewed after a rupture, whereas the easily-renewed epithelium presents no definite channel. It is not impossible, again, that a cinclis of this kind, once burst open, may retain the channel formed in the epithelium by rupture and regularise it.

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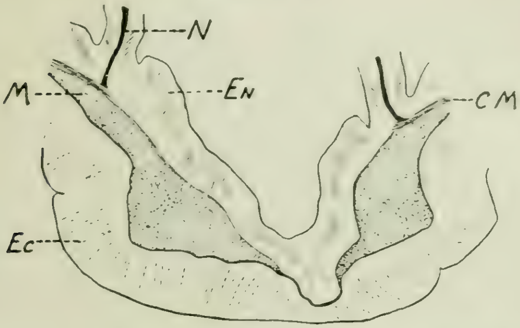
[1] It may be noticed that in this figure (Pl. 22, fig. 1) there is a little bridge of endoderm crossing the internal cavity of the cinclis. This is due to the fact that some cinclides have little lips of endoderm on their inner sides, and in this particular section the overhanging edge of one of these lips has got cut across. In this figure, as in Text-fig. 8, it will also be seen that the two cinclides from which these figures were drawn are only partly evaginations of the endoderm, and appear to be partly also ectodermal invagination. They are really more evaginations than they appear to be at first sight, because externally directed papillæ of mesoglœa happen to flank the opening of the cinclis and give rather a false impression when more of the parts surrounding the cinclis cannot be seen.

TEXT-FIG. 9.



Section of cinclis of Sagartia miniata. Lettering as on Text-fig. 2, p. 450. Also *N.* Mesentery.

There is one point which will be affected by the notes given above. Carlgren included, tentatively and with a query, in his diagnosis of the sub-families Metridiinæ and Sagartiinæ the character that in the former the cinclides are chiefly endodermal evaginations, and in the latter chiefly ectodermal invaginations. But since it is now seen that both kinds may occur in one animal, not to speak of one species or one genus, that character evidently cannot be used as a family one. The cinclides described by Carlgren for S. viduata are more like those characteristic of the lower zone in S. miniata than the others, but are not identical, though they are ectodermal invaginations. The cinclis figured by Hertwig for Calliactis parasitica (Sagartia parasitica) looks most like an outpushing of endoderm.

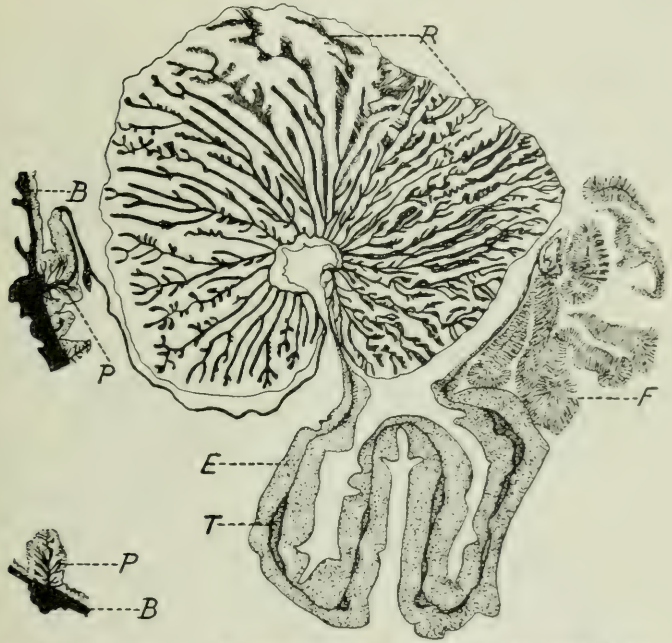
V. Discussion of Characters to be Used in Classification.

In choosing a series of characters for use in the discussions which follow I have selected those which appear to be the most important and those most generally distributed among the forms with which this paper deals—forms with mesoglœal sphincters. I have eliminated characters of merely generic importance and liable to occur anywhere. It is of course impossible to draw a hard-and-fast line between characters which are and are not of sufficient importance to rank as family characters, but I have made my list as fairly as I can.

I have been considerably impressed during the course of my work with the importance of one of the characters affecting the mesenteries. In many forms there is a marked differentiation of the mesenteries into two main types. I am applying to these types throughout the course of this paper the terms *macrocneme* and *microcneme*. I regret that it is necessary to introduce new terms, but I do not feel that it would be wise to risk confusion by not doing so. There are two words already in use—*macromesentery* and *micromesentery*—which would serve my purpose if taken in a certain sense, but I have noticed that different authors mean different things when they use these words, and have therefore introduced new ones to make my meaning clear. By a *macrocneme* I mean a large, perfect mesentery bearing a well-developed retractor muscle (usually "circumscribed" and consequently reniform in section), a gonad, and a well-marked filament; these in addition to a parietal muscle as a rule—generally the latter is very definite. A *microcneme* varies in the exact details of its development in different cases, but is typically reduced to little more than a narrow lamella bearing parietal musculature; as a rule it is not perfect, bears no gonad, no retractor muscle and no well-developed filament. The contrast between a typical macrocneme and a typical microcneme is well brought out by Text-figs. 11 (macrocneme of Phellia phassonesiotes) and 10 (microcneme of P. phassonesiotes), which are both drawn to the same scale.

It will be evident that if an anemone possesses a number —say six pairs—of macrocnemes, and all its other mesenteries are microcnemes, the distinction between the two is

Text-figs. 10 and 11.

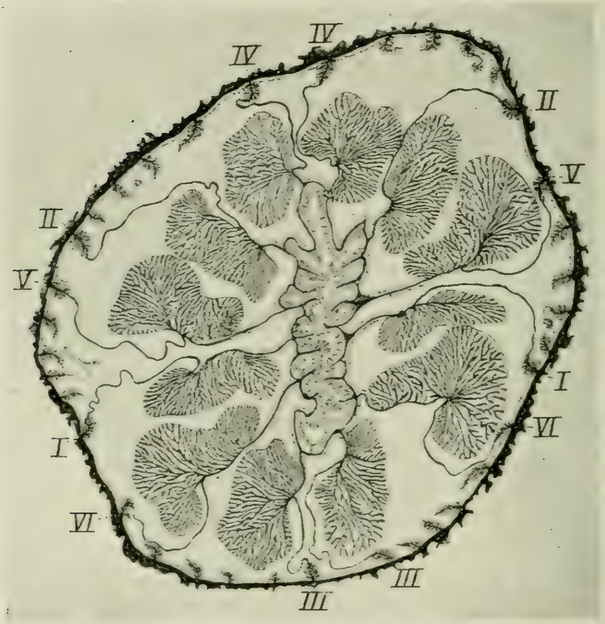


Text-fig. 10 (Below).—Microcneme of Phellia phassonesiotes. Text-fig. 11 (Above).—Macrocneme of Phellia phassonesiotes. Both drawn to same scale. *B.* Body-wall. *E.* Endoderm. *F.* Mesenterial filament. *P.* Parietal muscle. *R.* Retractor. *T.* Testis.

one of the most striking and important features of that form. Text-fig. 12 shows a good example of this. It is a transverse section of Phellia phassonesiotes, and shows how the macrocnemes fill up almost the whole cœlenteron, whilst the microcnemes are insignificant.

One must add the qualification that in Diadumene schilleriana (Pl. 22, fig. 12) (see p. 521) some of the microcnemes of some *individuals* may develop unusually and produce gonads and retractors. This means that in such individuals the distinction of the mesenteries into macro- and microcnemes is partially lost (see p. 499). Apparently

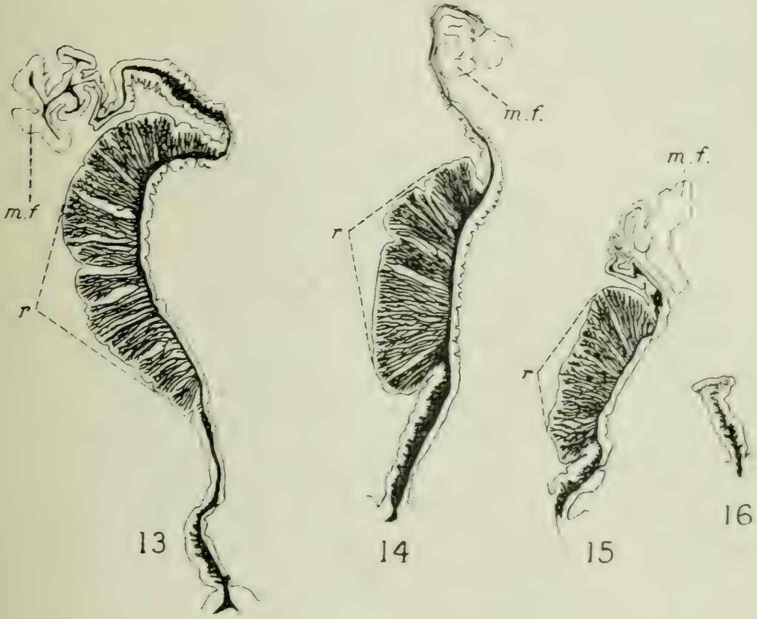
Text-fig. 12.



Transverse section of body of Phellia phassonesiotes.

it is not fully marked in this species in any case, although often the macrocnemes alone are fertile and always are alone perfect. The existence of this form, which is intermediate between those in which the division into macro- and microcnemes is fully marked and those in which no such distinction exists, does not alter the main fact of the usefulness of the presence or absence of this distinction as a classificatory character. There are many useful characters between which intermediates exist. Speaking generally, however, microcnemes are very small and at most bear a small filament and an acontium, together with an insignificant vestige of longitudinal muscle, or they may be rather broad lamellæ, but devoid of filament, etc. As an exceptional thing a few microcnemes in Pelocœtes (Pl. 22, fig. 13) may develop gonads, filaments, or retractors, but here the distinction into macro- and microcnemes is very definite (see Text-fig. 28), and as a rule the latter are rudimentary. The number of the macrocnemes is in most cases a stable and reliable character. It so happens that most of the forms here dealt with have six pairs of macrocnemes only, but one of them has twelve pairs, and in

Text-figs. 13–16.

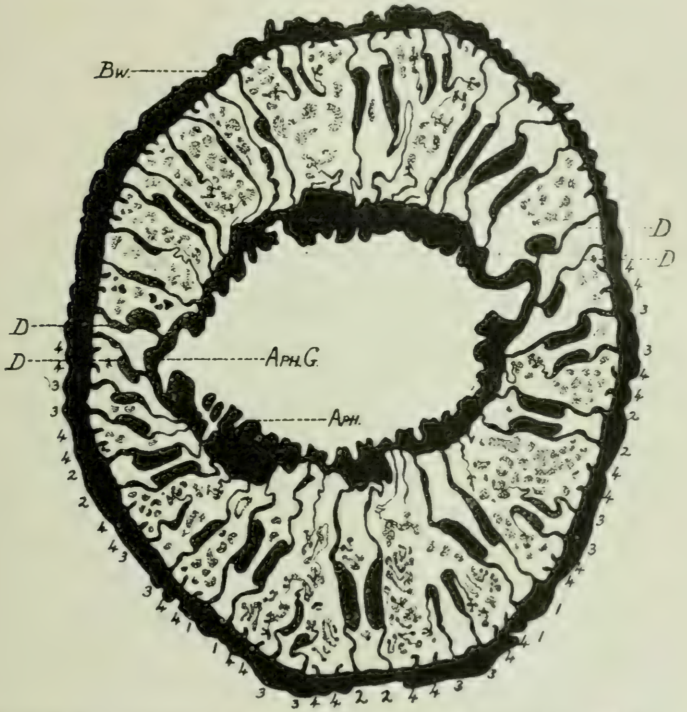


Mesenteries of Sagartia miniata, one mesentery from each cycle, of cycles 1–4. All drawn to same scale. *m.f.* Mesenterial filament. *r.* Retractor.

other families not under immediate consideration there are other numbers.

In species the mesenteries of which are not differentiated into macro- and microcnemes, it is found that the mesenteries of the first cycle may only differ from those of the second cycle by being a little larger, the second-cycle mesenteries differ from those of the third cycle in the same way, and so on—that is to say, there is a more or less graded series from the oldest cycle downwards and the primaries are not the only mesenteries that are really well developed. The mesenteries of *all* the older cycles bear well-developed retractors and filaments, the sizes of these diminishing as we pass from older cycles to younger ones. Text-fig. 19 shows several mesenteries from one of these forms (Actinauge richardi). It includes one primary, one secondary and one tertiary pair of mesenteries, all provided with filament and retractor and exhibiting a graduation in size. The fourth cycle here is still quite small. Text-figs. 13–16 show four mesenteries, one from each of the first four cycles, of Sagartia miniata, and bring out the gradation very well. All four figures are drawn to same scale. Among forms of this nature the retractors are typically diffuse, only rarely circumscribed. Two types of diffuse retractor are illustrated in Text-figs. 20 and 21, one of them more perfectly diffuse than the other. In this class of anemones the number of mesenteries which reach and join the actinopharynx—i.e. are perfect—may be six pairs only or may be many pairs. Here again there is not an absolutely clear-cut distinction, because a few species in which the standard number of perfect pairs is six show a tendency to develop seven or eight or even a few more perfect pairs (i.e. one or two more than the six primaries); and in a few species belonging to genera with a higher standard number of perfect mesenteries than six pairs the total number may not be *many* more than six. Nevertheless, to divide forms into those with six pairs perfect on the one hand and those with more than six (usually twelve or more) on the other, is on the whole a useful and reliable distinction if used as one character among others, and fits in very well with what we may suppose the evolutionary history of the group to have been (see p. 487). The contrast between a typical form with six and a typical form with more than twelve

TEXT-FIG. 17.



Transverse section of body of Sagartia miniata. *APH.* Actino-pharynx. *APH.G.* Actinopharyngeal groove. *BW.* Body-wall. *D.* Directive mesentery. Numbers indicate mesentery-cycles.

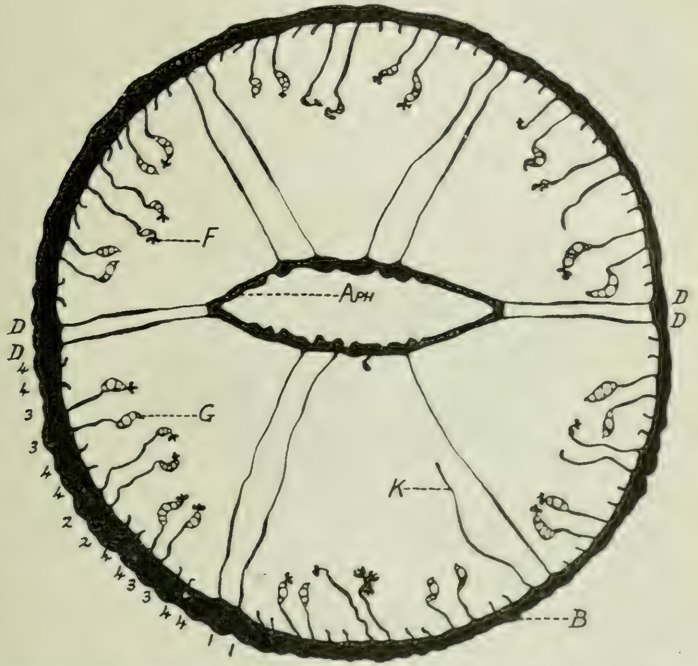
pairs of perfect mesenteries is well shown by Text-figs. 18 (transverse section, Calliactis parasitica) and 17 (transverse section, Sagartia miniata), which also bring out the absence of any distinction into macro- and micromesenteries. In Text-fig. 17 most of the mesenteries of cycles 1 to 3 are seen to be perfect, and those which do not actually join the actinopharynx in the figure do so in sections cut at a slightly higher level. Cycles 1 and 2 are almost indistinguishable at this level; cycle 3 is smaller, but all three cycles bear strong diffuse retractors (shown as black thickenings of the mesenteries), and their filaments and acontia are seen occupying the exocœls and endocœls as dotted patches.

In Text-fig. 18 the primary mesenteries are certainly much *wider* than the others, but this particularly marked width is only found at the level of the actinopharynx where they stretch out to join it, and it is clearly seen that they have no strong musculature like the macrocnemes of Phellia, nor are they fertile. On the other hand, in this figure the mesenteries of cycles 2 and 3 bear weak retractors, filaments and gonads. In both Text-figs. 17 and 18 the fourth-cycle mesenteries are small at the level sectionised, being the youngest cycle in evidence at that level. In forms like this the mesenteries of the youngest cycle (sometimes even more of the youngest ones) are liable to remain small, and may sometimes be practically in the condition of *all* the imperfect mesenteries or microcnemes of a form where the distinction into macro- and microcnemes prevails. On the other hand they may become important as gonad-bearers.

In these forms which have lost the distinction into macrocnemes and microcnemes, the distribution of gonads is variable. They may be borne by all the mesenteries, or by all the older cycles, or the primary mesenteries may be sterile. In some cases, even, all but the *youngest* mesenteries are sterile. The distribution of reproductive organs is too variable within this group to be of any general use as a family character, but there are two well-marked families in which the gonads (Text-fig. 18) adopt one or other method of appearance exclusively. These two groups are the Chondractiniidæ and Sagartiidæ as limited on pp. 533 and 544. For further remarks on gonads see p. 517.

To turn to another character, most frequently anemones have the longitudinal muscle-layer of the tentacles borne upon more or less slender processes of the mesoglœa, which project outwards into the ectoderm and support the actual muscle-fibres. This gives rise to a very characteristic appearance in a transverse section of a tentacle (or of the oral disc, the radial musculature of which generally resembles the longitudinal musculature of the tentacles), the outer edge of the mesoglœa appearing fringed with little dendrites. The size, form and mode of branching of the latter varies according to species. Pl. 22, fig. 9, shows a typical example from Sagartia miniata.

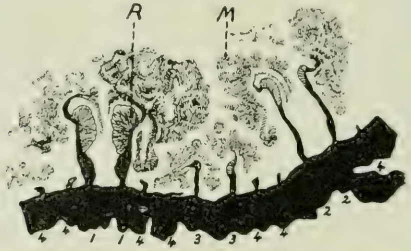
TEXT-FIG. 18.



Transverse section of body of Calliactis parasitica. *APH.* Actinopharynx. *B.* Body-wall. *D.* Directive mesentery. *F.* Mesenterial filament. *K.* Interrupted perfect mesentery. Numbers indicate mesentery-cycles.

In some genera, however, most of which on other grounds appear to be advanced, the longitudinal musculature of the tentacles and radial musculature of the disc have become entirely embedded in the mesoglœa. It no longer has the form of branching processes bearing the fibres, but becomes a network of cavities the walls of which support the fibres (Pl. 22, fig. 8), or else the fibres merely lie in bundles in the mesoglœa. This state of affairs is obviously an advance on that in which the musculature is ectodermal, and is of a certain classificatory importance. There is not, it is true, any single family in which *all* the members present this feature, but in the Paractidæ (in the strict sense, as defined on p. 548) it is so far the rule as to be of value in determining the relationships of the family. In a few cases the ectodermal type shows a partial sinking into the mesoglœa.

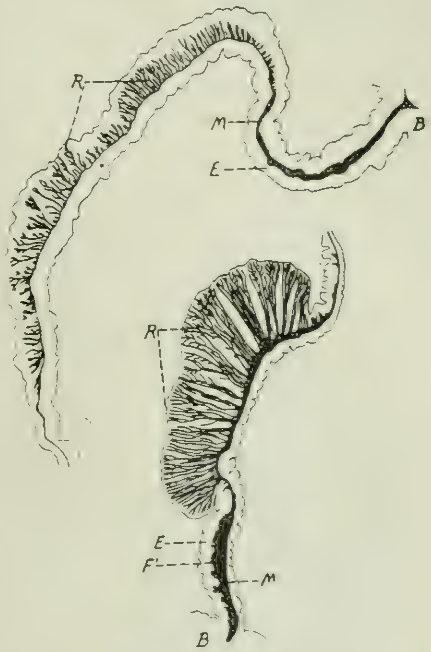
TEXT-FIG. 19.



Transverse section of a part of body of Actinauge richardi showing part of body-wall and some mesenteries. *M.* Mesenterial filament. *R.* Retractor. Numbers indicate mesentery-cycles.

In some anemones the body is "all in one piece"—that is, not distinguished into definite regions. But in other cases three more or less well-marked zones are differentiated. These are seen at their best in Edwardsia, where the greater part of the body, or *scapus*, is marked off not only from the more delicate oral extremity (*capitulum*) which bears the tentacles, but also from the rounded vesicular aboral extremity or *physa*. In Phellia this distinction into three regions is also well marked. The scapus is corrugated and covered by a definite cuticle, and the more delicate capitulum is free from cuticle. Here the aboral end is really more a physa than anything else. Although it may be adherent it is more or less inflatable and is hardly as developed a pedal disc as is possessed by higher forms. In some other forms where a more definite pedal disc is present, the column may still be divided into a lower scapus provided with a cuticle as a rule, and an upper portion devoid of it. Although this upper portion does not exactly correspond with Gosse's meaning of the term "capitulum," since it is not particularly delicate in many cases, it would be rather confusing to give it another name, especially as the word "capitulum" has often been applied to it already. When a capitulum is present it can generally be introverted more or less into the scapus, and in such forms as Phellia and Edwardsia part of the scapus can be introverted also. I am using this series of characters as one among others in defining those families all the members of which conform to one type or the other. The basal extremity of the anemone may vary a good deal. Either it may be rounded and vesicular, as in Edwardsia and Halcampa—and then it constitutes a true physa—or it may form a definite and permanent pedal disc for adhesion, the

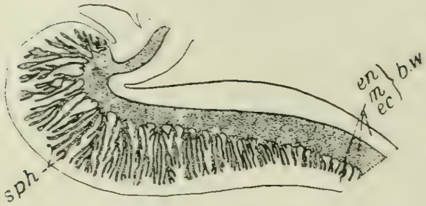
TEXT-FIGS. 20 AND 21.



Text-fig. 20 (Above).—Mesentery of Aiptasia couchi. Text-fig. 21 (Below).—Mesentery of Sagartia miniata. *B*. Body-wall. *E*. Endoderm. *F'*. Short mesoglœal processes for muscle. *M*. Mesoglœa. *R*. Retractor.

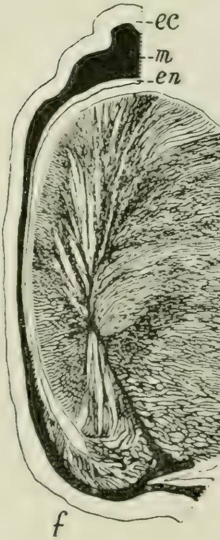
pedal disc in some cases becoming concave and enclosing mud instead of adhering. There are, however, intermediates between true physa and true pedal disc. Some Phellias possess what is practically a physa, but may adhere. Further reference to this will be found at various points.

Text-fig. 22.



Sphincter of Bolocera longicornis. *bw.* Body-wall. *ec.* Ectoderm. *en.* Endoderm. *m.* Mesoglœa. *sph.* Sphincter.

Text-fig. 23.



Sphincter of Epiactis novozealandica. *f.* indicates the fosse. Remainder as in Text-fig. 22.

The Actiniina (i.e. those anemones which are outside the Stichodactylina, Edwardsiaria, Ceriantharia, and Zoanthinaria) seem to fall into two fairly well-marked groups according to the character of the marginal sphincter of the body-wall. The first group has this sphincter, if present, endodermal—that is to say, its fibres are supported on processes of the mesoglœa which project into the endoderm. The endodermal sphincter may be diffuse (Text-fig. 22) or very much aggregated and circumscribed (Text-fig. 23). In the second group, which is the one directly dealt with in this paper, the sphincter has sunk entirely into the mesoglœa, and the fibres become supported by the walls of cavities or lie in bundles in the mesoglœa (Pl. 22, fig. 3).

In the group where the mesoglœal sphincter is the rule, it is interesting to note that when special stinging organs are developed they are acontia, and not acrorhagi, etc.; nor are vesicles or pseudo-tentacles found within the group. Such structures as acrorhagi, vesicles and pseudo-tentacles occur freely among the forms with an endodermal sphincter (though of course by no means universally), and acontia do *not* occur in these forms.[1] These are of course more differences of tendency than anything else, but they are interesting.

There are a few genera which possess acontia but have no sphincter at all. These I am including in the following discussions, with the forms which *have* the mesoglœal sphincter, because they agree with them in all other ways, and a study of them individually (carried out on p. 508) shows that they are clearly related to mesoglœal sphinctered forms.

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[1] With regard to the above paragraph, I would point out that although certain species have been recorded which do not agree with the statements I have made, the records are not in any case sufficiently certain, and need confirmation. If it should be found in the end that these records are accurate, it will involve certain modifications of my views, though not very fundamental ones. But for the present I prefer to leave doubtful genera out of account. The cases I refer to are those of Nemactis (a form said to have both acontia and acrorhagi), Ophiodiscus (please see note on this genus on p. 560) and certain genera described by Danielssen as possessing acontia and endodermal sphincters. Doubt has been thrown upon the anatomical work of the latter author, in some cases at all events, and it needs confirmation. One of the forms he describes as possessing acontia, and an endodermal sphincter is considered by Carlgren as a synonym of Stomphia churchiæ, in which case it has neither. If Aiptasia mutabilis has really an endodermal sphincter it is possible that there is a special explanation of the endodermal sphincter in that particular case, as I shall show later on (p. 510). But as far as I can make out from Simon's description, it seems to me that what he is describing is not the true sphincter, but a secondary concentration of the endodermal circular muscle such as McMurrich and Hertwig have described in Aiptasia sp. ('Proc. Acad. Nat. Sci., Philad.,' 1889) and Leiotealia nymphæa. In both these species there is a true sphincter as well as and apart from the concentration of endodermal circular muscle in question, and the latter seems to be constricting the column to some extent, and is perhaps only temporary. It is not improbable that when living anemones constrict their bodies the endodermal circular muscle may form a temporary concentration. If this is the case in A. mutabilis it means that there is no true sphincter, which brings it into line with other Aiptasia-like forms.

Presence or absence of acontia and presence or absence of cinclides are characters which will be used, but I need not say more about them here as they have been fully discussed above. Generally speaking, we find a correlation between cinclides and a delicate body-wall always free from cuticle.

VI. A NEW METHOD OF GROUPING THOSE FORMS WHICH POSSESS EITHER ACONTIA OR A MESOGLŒAL SPHINCTER OR BOTH THESE CHARACTERS.

In studying various groups of the animal kingdom one is struck with the fact that it is possible to follow up, in some given group, a certain number of lines of evolution affecting certain organs or aspects of the animals in the group. It may happen that there are two or more alternative classifications of the group, which may express different lines of evolution, but which overlap and are not much correlated. The Lamellibranchs are a case in point. Attempts have been made to classify them on the basis of—

(1) Gill structure.
(2) Adductor muscles.
(3) Hinge-lines and teeth.
(4) Pallial lines and siphons.

It is possible to trace out what seem to be lines of evolution affecting each one of these aspects of the group, but when each of them is embodied in a separate classification there is overlapping and a certain lack of coherence. Each classification expresses the history of one organ or set of organs, but gives little clue to the probable history of the group as a whole.

It is a case which shows clearly that an ideal classification—that is, a classification expressing the history of the organisms of a group considered as wholes, cannot be built up around single characters, but must start upon the basis of the aggregate of the most important features of the animals to be classified—that is to say, the sum of the more important characters should be used. Such a classification might hope to express the natural relationships of the whole animals as well as expressing the group-history as far as known, rather than the relationships and history of one isolated part of the structure.

This point of view is not only borne out but necessitated by the Mendelian discoveries, which show that any character or set of characters may have an independent hereditary history in the same series of organisms.

In the case of the Actiniina the application of this principle is badly needed. By a detailed study of the occurrence of acontia and of the mesoglœal sphincter in this group, Bourne has already shown that both these characters are too widely distributed through the group to imply any very close genetic relationship between the various forms possessing them, although, as I hope to show, in these particular cases there seems to have been a common ancestor. He has shown that we must dispose, once for all, of the idea that because two given forms possess acontia they must therefore be united in a single family. I have indicated this from my own point of view in my discussion of acontia and cinclides, and have endeavoured furthermore to show that the cinclis-character is not bound up with the acontium-character, and may be used independently. Whatever character be taken, it will be found that if it is attempted to build up families upon one or two characters only, the result is to bring together unrelated forms. No one would dream of uniting Ophiodiscus and Halcampa on the strength of the mesoglœal sphincter, or Actinauge and Alicia because both possess only six pairs of perfect mesenteries.

I hope to show that the families "Sagartiidæ" and "Paractidæ," as at present understood, are in reality assemblages of more or less unrelated forms; that some genera belonging to one family are nearer certain genera of the other family than to those genera now classed with them; and that if we treat the Sagartid and Paractid genera as an unclassified series we can build up from among them, on the basis of the sum of the more important characters, a number of very natural groups.

At this point, however, it is necessary to add a caution about the application of the sum-of-the-character principle. A certain amount of discrimination must be used in applying it. It may not be universally applicable, and it cannot in any case be used in a mechanical way like an arithmetical measure. The scheme will only give profitable results if a discrimination is made between those characters which are of primary importance and those which are of lesser value.

Characters of the latter class are those which are of value in distinguishing genera from each other, but cannot be introduced in considering families. Some of them are features which may have been independently acquired by two or more forms which are shown on the sum of more fundamental characters to be unrelated, in correlation with some similar but special mode of life (such as retirement to deep water) in the two cases. Opinions will naturally differ as to which *are* the most important characters, but in dealing with these anemones I have selected those which seem the most fundamental and least open to objection.

First of all let us consider the relationships of certain groups of genera.

1. Marsupifer[1] and Phelliomorpha share the following nine chief characteristics:

(1) Column divided into scapus and capitulum.
(2) Scapus provided with cuticle.
(3) Mesenteries divided into macro- and microcnemes.
(4) Number of macrocnemes limited to six pairs.
(5) Pedal disc present.
(6) Sphincter mesoglœal.

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[1] With reference to Marsupifer I propose to leave out of account the possession of brood-pouches by the female, as a special development, not of more than generic value at most for classificatory purposes. This character is one liable to occur anywhere in any family—cf. Epiactis. The double sphincter of Marsupifer, again, is a character peculiar to it as a *genus* only.

(7) Longitudinal musculature of tentacles ectodermal.[1]
(8) Acontia absent.
(9) Cinclides absent.

If we compare these genera with Phellia and Decaphellia, we find that the two latter genera have seven points (Nos. 1, 2, 3, 4, 6, 7, 9) in common with Marsupifer and Phelliomorpha, whereas the points in which the two pairs of genera differ from one another are only two in number, viz.—

(1) Phellia and Decaphellia have acontia, Marsupifer and Phelliomorpha have not.
(2) In Phellia and Decaphellia the base may be more or less a physa; in Marsupifer and Phelliomorpha a more definite pedal disc is present.

So far it is clear that Phellia and Decaphellia have so much in common with the other two genera that they must be considered as more or less related to them, though certainly not closely enough to be placed in one and the same family. Hitherto Phellia, etc., have been included in the Sagartiidæ and Marsupifer in the Paractidæ. It will therefore be advisable to examine their relationship to the typical forms of those families, i. e. Sagartia and its nearest relatives and Paractis and its nearest relatives.

The genera Sagartia, Cereus and Artemidactis have in common the following nine main features:

(1) No division of the mesenteries into macrocnemes and microcnemes—the cycles are graded.
(2) Number of perfect mesenteries exceeding six pairs usually exceeding twelve pairs.
(3) Adherent base present.
(4) Sphincter mesoglœal.
(5) Acontia present.
(6) Cinclides present.
(7) Cuticle absent, body-wall fairly delicate.
(8) Longitudinal muscles of tentacles ectodermal.
(9) Primary mesenteries fertile as well as others.

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[1] This is the rule. Rarely a *part* of the musculature is enclosed by anastomosis of the mesoglœal processes which project into the ectoderm.

Of these nine characters, Nos. 3 (more or less), 4, 5, 8 recur in Phellia and Decaphellia. Character 9 is not applicable to them, because although their primary mesenteries are fertile no others are, all the others being microcnemes. The Phellia group, then, has four characters in common with the Sagartia group, and five points of difference. But we have already seen that the Phellia group has seven features in common with Marsupifer, etc., so it is evident, not only on this account but also because of the large number of differences between the Phellia and Sagartia groups, that Phellia, etc., are more closely related to Marsupifer, etc. (hitherto classed as "Paractid"), than to Sagartia, etc. But since Phellia and Marsupifer, although fairly nearly related, are not near enough to each other (for the differences see above) to be classed in the same family, it is clear that Phellia and Decaphellia must be separated from the Sagartia group and classed as a separate family. Bourne has listed the characters of the Phelliidæ (**10**, p. 78) in a slightly different way, and has shown clearly that of thirteen characters especially distinguishing them, no less than ten are found also in Halcampa (which differs from Phellia in so far as it lacks acontia and cuticle and possesses cinclides). Of Bourne's thirteen characters only two (presence of acontia and a mesoglœal sphincter) are present in Sagartia, whereas Phellia and Sagartia show eleven points of difference. From this it is seen that Phellia has so much more in common with Halcampa than with Sagartia that it must be placed in a separate family from the latter, near the Halcampidæ. Bourne therefore claims family rank for the group containing Phellia and Decaphellia, and I have given additional evidence for the necessity of this by comparing them with Marsupifer and Phelliomorpha, which are their near relations on a different side from Halcampa. It now remains to compare Marsupifer, etc., with those forms most typical of the Paractidæ.

The genera Paractis, Cymbactis, Hormosoma, Actinostola, Catadiomene and Stomphia, the forms in question, share four of the characters listed on p. 470 for Marsupifer, etc. (Nos. 5, 6, 8, 9), but differ from the Marsupifer group as regards characters 1, 2, 3, 4 and 7, some of which are most fundamental characters affecting the mesenteries.

If, therefore, Marsupifer, etc., agree with Phellia, etc., as regards seven characters (and disagree in two), whereas they only agree with the typical Paractids in four and disagree in five respects, they have clearly more in common with Phellia than with Paractis, and cannot therefore be included in one family with Paractis. But since they are at the same time *sufficiently* different from Phellia to claim family distinction, they must be placed in a group of their own.

Another group of forms which will afford us a good example is that which contains the genera Diadumene, Pelocœtes. Phytocœtes, and Mena (Pl. 22, figs. 12, 13, Text-fig. 28). These four genera have seven important common characters:

(1) Mesenteries divided into macrocnemes and microcnemes.[1]

(2) Number of macrocnemes limited to six pairs.

(3) Acontia present.

(4) Cinclides present.

(5) A definite base present which may be reduced and physa-like.

(6) Body-wall delicate and devoid of cuticle.

(7) Longitudinal musculature of tentacles ectodermal.

Sphincter usually absent, but may be mesoglœal.

These forms (the Diadumene group) share characters 1, 2, 4, 6, 7 with Halcampa, from which they chiefly differ by the possession of acontia and a definite base; but even the latter may be reduced in connection with a burrowing life.

Secondly, the Diadumene group shares characters 1, 2, 3, 5 and 7 with the Phelliidæ (Phellia and Decaphellia), from which it differs in that its members possess cinclides but no cuticle and no division of the body into scapus and capitulum.

[1] This character being somewhat violated by Diadumene.

Thirdly, the Diadumenids share characters 3, 4, 5, 6, 7 with the Sagartia-Cereus-Artemidactis group, but differ from the latter fundamentally as regards the mesenteries.

From this it is evident that—

(1) These forms are as closely related to Halcampa and Phellia as to Sagartia, and cannot therefore be classed with the latter. (If they are carefully studied they really seem nearest of all to Halcampa.)

(2) Since they are equally related to three other different sets of genera, but not quite closely enough to any one of the latter to be included in the same family (see differences noted above) with them, they must obviously be established apart as a family distinct from either of the other three sets.

A last example may be taken by comparing the genera Actinoscyphia, Isoparactis and Paranthus with certain other groups of genera.

The three genera mentioned have the following seven important common characters:

(1) Primary mesenteries grade into secondaries, secondaries into tertiaries, etc., so that there is no division into macrocnemes and microcnemes.
(2) Number of perfect mesenteries limited to six pairs.
(3) Pedal disc present.
(4) Sphincter mesoglœal.
(5) Acontia absent.
(6) Cinclides absent.
(7) Longitudinal musculature of the tentacles ectodermal.

The Paractis group possess characters 1, 3, 4, 5, 6 of the above list, and a few of them also No. 7. The chief difference is that the Paractids have numerous perfect mesenteries, the Actinoscyphids only six pairs, added to which the majority of forms related to Paractis, including all those referred to above as its most typical relatives, have the longitudinal musculature of the tentacles embedded in the mesoglœa.

The large group of genera containing Actinauge, Chondrodactis, Hormathia, Paraphellia, etc.—i.e. the Chondractiniinæ of Haddon—possess *all* the characters above mentioned for Actinoscyphia, etc., except that they have acontia (which may be rudimentary).

Consequently Actinoscyphia and its relations have more in common (six features) with, and less to separate them from (one feature), the Chondractiniinæ than they have with the Paractids with which they have so far been classed. But the absence of acontia is enough to separate them from the Chondractiniinæ, and I have simply introduced the comparison to show that they cannot be included with Paractis, etc., when they are even more related to another set of forms than they are to that. So Actinoscyphia, etc., must also constitute a distinct family.

The above analysis brings out several facts. It shows that “Sagartiidæ”, and “Paractidæ” as they stand are both families containing a wide series of forms, the extremes of which are so much separated that it is impossible to place them in one and the same family on the strength of one or two resemblances only. It is quite imperative, for instance, to separate the Phellia group and the Diadumene group from Sagartia and its nearest relatives, and the Marsupifer group and the Actinoscyphia group from Paractis and its allies. It is also seen that some “Sagartids” (e.g. Phellia) are more closely related, considered on the sum of the principal features, to certain “Paractids” (e.g. Marsupifer) on the one hand, and to Halcampa on the other, than they are to other “Sagartids.” Clearly the families “Sagartiidæ,” “Paractidæ” and “Halcampidæ” are so much inter-related that they no longer represent an adequate classification, and must be broken up. We can no longer define two groups as “Actiniina possessing acontia” (“Sagartiidæ”) and “Actiniina possessing a mesoglœal sphincter and a pedal disc but no acontia” (“Paractidæ”), because that arrangement is now seen to be unnatural and insufficient.

If, therefore, we treat all the Paractid and Sagartid genera as a completely unclassified series for the moment, and apply to them the principle of the sum of the most important characters, we shall find that we can build them up into a number of eminently natural groups. Each of these groups will represent a collection of genera clearly defined on the basis of the possession of a sufficient number of the most important common characteristics, and as such will rank as a family. Each family will present at least six, generally seven or eight or nine, features of importance common to all its members. I will enumerate these families without further discussion, and comment upon them afterwards. In splitting up the "Sagartiidæ" and "Paractidæ," I have, of course, retained those two names for that group of genera which may be considered most typical in each case, i.e. Sagartia and its nearest relations in the first case, and Paractis and its closest relatives in the second.

1. DIADUMENIDÆ.

The genera Diadumene, Mena, Pelocœtes and Phytocœtes form this family, and have in common the following seven characters:

(1) Mesenteries divided into macrocnemes and microcnemes, the division not fully expressed in Diadumene.
(2) Number of macrocnemes limited to six pairs.
(3) Acontia present.
(4) Cinclides present.
(5) A definite base present which may be reduced and physa-like.
(6) Body-wall delicate and devoid of cuticle.
(7) Longitudinal musculature of tentacles ectodermal.

Sphincter usually absent, but may be very weak mesoglœal.

2. PHELLIIDÆ.

Genera: Phellia, Decaphellia, Halcampactis. Number of common characters, 8.

(1) Body divided into scapus, capitulum, and base or physa, which may adhere.
(2) Scapus provided with a cuticle.
(3) Mesenteries divided into macro- and microcnemes.
(4) Maximum number of macrocnemes six pairs.[1]
(5) Capitulum and upper third of scapus can be introverted.
(6) Acontia present.
(7) Cinclides absent.
(8) Longitudinal musculature of tentacles ectodermal or rarely partly enclosed by anastomosis of ectodermal processes of mesoglœa.

Sphincter mesoglœal in Phellia and Decaphellia, possibly also in Halcampactis; or if not, absent.

3. MARSUPIFERIDÆ.

Genera: Marsupifer and Phelliomorpha. Number of common characters, 9.

(1) Body divided into scapus and capitulum.
(2) Scapus provided with a cuticle.
(3) Mesenteries divided into macrocnemes and microcnemes.
(4) Number of macrocnemes limited to six pairs.
(5) Pedal disc present.
(6) Sphincter mesoglœal.
(7) Acontia absent.
(8) Cinclides absent.
(9) Longitudinal musculature of tentacles ectodermal.

4. METRIDIIDÆ.

Genera: Metridium, Calliactis, Adamsia, Aiptasia, Aiptasiomorpha, Heteractis, Bartholomea. Number of common characters, 7.

(1) Cycles of mesenteries grading into each other, so no division into macrocnemes and microcnemes is present.
(2) Number of perfect mesenteries limited to six pairs or a very few more.

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[1] Or in exceptional instances a very few more.

(3) Pedal disc present.
(4) Acontia present.
(5) Cinclides present.
(6) Cuticle absent; body-wall usually more or less delicate.
(7) Longitudinal musculature of tentacles wholly or partly ectodermal.

Sphincter mesoglœal except in Aiptasiomorpha and Bartholomea, where it is absent.

5. CHONDRACTINIIDÆ.

Genera: Hormathia, Actinauge, Chondrodactis, Paraphellia, Sagartiomorphe, Leptoteichus. Number of common characters, 8.

(1) Cycles of mesenteries grading into each other, so no division into macrocnemes and microcnemes is present.
(2) Number of perfect mesenteries limited to six pairs or a very few more.
(3) Pedal disc present.
(4) Acontia present.
(5) Cinclides absent.
(6) Longitudinal muscles of tentacles partly or wholly ectodermal.
(7) Primary mesenteries always sterile.
(8) Sphincter mesoglœal.

6. ACTINOSCYPHIIDÆ.

Genera: Actinoscyphia, Paranthus, Isoparactis. Number of common features, 7.

(1) Cycles of mesenteries grade into one another, so there is no division into macrocnemes and microcnemes.
(2) Number of perfect mesenteries limited to six pairs.
(3) Pedal disc present.
(4) Sphincter mesoglœal.
(5) Acontia absent.
(6) Cinclides absent.
(7) Longitudinal musculature of tentacles ectodermal.

7. SAGARTIIDÆ (in a much more restricted sense than hitherto).

Genera: Sagartia, Cereus, Artemidactis. Number of common characters, 9.

(1) Cycles of mesenteries grading into one another, so that no division into macrocnemes and micronemes is present.
(2) Number of perfect mesenteries exceeding six pairs, usually exceeding twelve pairs.
(3) Primary mesenteries fertile as well as others.
(4) Cuticle absent; body-wall fairly delicate.
(5) Cinclides present.
(6) Acontia present.
(7) Sphincter mesoglœal.
(8) Longitudinal musculature of tentacles ectodermal.
(9) Pedal disc present.

8. CHORIACTIDÆ.

The genus Choriactis stands alone. Perhaps Mitactis and some other forms will go with it when better known. Its chief characteristics are:

(1) Cycles of mesenteries grading into one another so that no division into macrocnemes and microcnemes is present.
(2) Number of perfect mesenteries considerable.
(3) Pedal disc present.
(4) Sphincter mesoglœal.
(5) Acontia present.
(6) Cinclides absent.
(7) Wall may be thick; no cuticle.
(8) Longitudinal musculature of tentacles ectodermal.

9. PARACTIDÆ (in a much more restricted sense than hitherto).

Genera: Paractis, Cymbactis, Stomphia, Hormosoma, Alloactis, Actinostola, Catadiomene, Polysiphonia, Ophiodiscus, Sicyonis, Antholoba, Parantheoides, Tealidium. Number of common features, 6 and a seventh not quite universal tendency.

(1) Cycles of mesenteries grading into each other, so no division into macrocnemes and microcnemes is present.
(2) Numerous mesenteries perfect.
(3) Sphincter mesoglœal.
(4) Acontia absent.
(5) Cinclides absent.
(6) Pedal disc present.

In Paractis, Cymbactis, Stomphia, Hormosoma, Actinostola, Catadiomene, Polysiphonia, Ophiodiscus, Sicyonis, the longitudinal musculature of the tentacles and radial musculature of the oral disc is entirely embedded in the mesoglœa. In Antholoba and Alloactis it is partially so.

It will be observed that in all the families enumerated above there are forms possessing a mesoglœal sphincter and that in six of the nine all forms have it. In the three families where there are exceptions these exceptional genera agree in all other respects with the other members of their families which *do* possess the sphincter and are bound to be classed with them on the sum of the characters. We may, therefore, fairly consider the mesoglœal sphincter as a character common to all nine families which has been lost in a few special cases, probably in correlation with some special form of life. This probability is more fully discussed in connection with the individual sphincterless genera on p. 508. *The presence of the mesoglœal sphincter is the only character common to all families,* apart from the fact that some sort of definite base is usually developed, and even this may be physa-like.

The sphincter apart, it will be noted that there are a number of other characters, presence or absence of which determine the limits of the groups. Some of these occur in such a regular manner that it will be worth while to consider them in tabular form. The characters to which I refer are :

(1) Presence or absence of acontia.
(2) Presence or absence of cinclides.

	Acontia present. Cinclides present.	Acontia present. No cinclides.	No acontia. No cinclides.
Mesenteries divided into macrocnemes and microcnemes. Number of macrocnemes typically six pairs at most.	Diadumenidæ. Diadumene. Pelocœtes. Phytocœtes. Mena. 7.	Phelliidæ. Phellia. Decaphellia. Halcampactis. 8.	Marsupiferidæ. Marsupifer. Phelliomorpha. 9.
Mesenteries *not* divided into macrocnemes and microcnemes. Number of perfect mesenteries six pairs or rarely a very few more.	Metridiidæ. Metridium. Calliactis. Aiptasia. Aiptasiomorpha. Adamsia. Heteractis. Bartholomea. 7.	Chondractiniidæ. Actinauge. Hormathia. Chondrodactis. Leptoteichus. Sagartiomorphe. Paraphellia. ? Phelliactis. 8.	Actinoscyphiidæ. Actinoscyphia. Paranthus. Isoparactis. ? Lilliella. 7.
Mesenteries *not* divided into macrocnemes and microcnemes. Number of perfect mesenteries exceeding six pairs, usually exceeding twelve pairs.	Sagartiidæ. Sagartia. Cereus. Artemidactis. 9.	Choriactidæ. Choriactis. ? Mitactis. 8.	Paractidæ. Paractis. Hormosoma. Stomphia. Actinostola. Ophiodiscus. Polysiphonia. Antholoba. Parantheoides. Tealidium. Sicyonis. Catadiomene. Cymbactis. Alloactis. 6.

In this table the number in the corner of each square is the number of characters which the members of the family in that square have in common.

(3) Presence or absence of a sharp division of the mesenteries into macrocnemes and microcnemes.

(4) Limitation of number of macrocnemes, when macrocnemes are present, to six pairs, or absence of this limitation.

(5) Presence or absence of any considerable number of perfect mesenteries over and above the six primary pairs in forms where the distinction between macrocnemes and microcnemes has been lost.

In the above table one square is given up to each family; the characters printed at the head of each of the three vertical columns apply to all three of the squares in that column. The characters printed at the side of the table apply to the three families on a horizontal level with them. So one can arrive at the four main points about any one family by combining the two points at the head of the vertical column containing that family with the two points on a level with the square occupied by it.

From this it will be seen that—

(1) Among those three families in which acontia and cinclides occur all three kinds of mesenterial development are found: the first, where there are six pairs of macrocnemes and all other mesenteries are microcnemes; in the second, where there is no distinction into macro- and micronemes, but where only six pairs are perfect, in the Metridiidæ; and the third, similar to the second but with numerous perfect mesenteries, in the Sagartiidæ.

(2) Among the three families in which there are acontia but no cinclides, *the same three types of mesenterial arrangement occur*—the first in the Phelliidæ, the second in the Chondractiniidæ, and the third in the Choriactidæ.

(3) The same remarks apply to the three families in which acontia and cinclides are both absent. Here the first type of mesenterial arrangement is found in the Marsupiferidæ, the second in the Actinoscyphiidæ, and the third in the Paractidæ.

4) If we were to start from another point of view we should find among those forms which possess the division of

mesenteries into macro- and microcnemes some forms with acontia and cinclides (Diadumenidæ), some with acontia and no cinclides (Phelliidæ), and some with neither the one nor the other (Marsupiferidæ). If we took, again, either those forms in which there is no division into macro- and microcnemes, but which have six pairs of mesenteries alone perfect, or those in which numerous pairs are perfect, we should find that in each case some of them had acontia + cinclides, some acontia and no cinclides, and some no acontia and no cinclides.

This shows that if we begin to build up families founded on mesentery-characters only, each one will include forms with acontia and cinclides, forms with acontia only, and forms with neither one nor the other, which is out of the question. Again, it is equally out of the question to found families on acontium- and cinclis-characters only, since then each family would include forms presenting fundamentally different types of mesenterial arrangement. It is therefore evident that we must use, not mesentery-characters only nor acontium-and-cinclis-characters only, but both in combination. Other characters may be brought in as well.

In this way the conclusion is reached that the nine sets of forms enumerated above as groups of genera clearly defined on the basis of the sum of their most important characters, and *not subordinate to other modes of grouping,* rank as families. The possibility of including all nine groups in *one* family, defined by the sole common character of the possession of a mesoglœal sphincter, thus bringing together a host of forms with widely separated extremes, is too far from the mark to need discussion.

The nine families thus established are more fully defined on pp. 520–564. The discussion of their probable evolutionary history which follows will, I hope, give the arrangement an intelligible meaning.

It is, perhaps, worth noting that a number of these nine families have been in existence already as subfamilies. These are the Phelliidæ, Metridiidæ, Chondractiniidæ and Sagartiidæ in the new sense. These have long been recognised as natural groups, but apart from them the allocation of forms has been unsatisfactory.

Before going on to discuss the evolution of the forms included in these families, it is necessary to speak of a form which I have not included in any one of them, and which I only found after most of the work for this paper was done. This form is one having the general appearance of a large Phellia, and it is near Carlgren's Isophellia; but till more is known of both Isophellia and of my form, it is not possible to assume their identity, and I have given mine the name Flosmaris. A short description of it will be found on pp. 441–443, and I give here a generic definition of it so that there will be a proper basis for discussion.

Flosmaris, n. gen.

Basal disc present. Body divided into a long scapus and a short capitulum; lower part of scapus may be very thin-walled. Main part of scapus with numerous small papillæ which attach sand-grains. Capitulum fairly smooth, devoid of sand, its margin tentaculate. Tentacles simple, their longitudinal musculature ectodermal. Sphincter rather small, confined to upper part of capitulum. Mesenteries sharply divided into macrocnemes and microcnemes. Twelve pairs of fully developed macrocnemes bearing gonads, circumscribed retractors and filaments, and all perfect. The other mesenteries are microcnemes and consist chiefly of a parietal muscle. Acontia present. Cinclides probably absent.

Species: F. phellioides, n. sp.

If reference be now made to the table of squares on p. 481, it will be seen that the squares actually shown in the table do not represent every *possible* combination of the characters placed at the heads and sides of the columns. They represent, however, every combination of these characters which is actually represented by hitherto-described anemones, with the one exception of Flosmaris.

If one were to construct a table showing every possible combination of the characters used, the number of squares would be not nine but sixteen. We should have one additional vertical column for forms with cinclides but no acontia, and one additional horizontal column for forms with the mesenteries divided into macrocnemes and microcnemes, but with the number of macrocnemes twelve or more pairs instead of six pairs.

I have not actually included the additional squares which would result from adding these two other possible columns, because as yet too little is known of any possible forms which might fill them to make it safe to do so. But Actiniaria are unexpected creatures, and one finds that new combinations of characters *do* turn up among them. It is therefore not at all impossible that some forms may occur and be discovered in the future which will fill some or all of the squares not covered by the nine already filled. *If this should happen, I claim that on the same grounds which make each of the nine known families valid, it will be necessary to erect further families until the limit of the possible combinations of the four characters in question be reached.* If forms representing *new* combinations should simply be allocated to the one of the nine already known families to which they happen to be nearest, it would eventually widen the limits of those families too much, and cause confusion, and each family would cease to represent an evolutionary stage or a natural group. It may be objected that a great multiplication of families is involved if all combinations are to have their due; but this is not really a valid criticism, because the arrangement of having a fairly large number of comparatively small and exactly-defined families is actually a very convenient one, and facilitates identification, quite apart from the fact that it gives more chance of having homogeneous and natural groups and representing relationships of whole animals and not parts of animals by them.

To apply all this to Flosmaris. *This species does actually represent one of the hitherto unrecorded combinations not included in the table.* It represents the combination of acontia and (probably) no cinclides with the division of the mesenteries into macrocnemes and microcnemes, but with twelve pairs of macrocnemes and not six pairs as in the Phelliidæ. It comes fairly close to Phellia of course, but one may claim separation for it on the same grounds as those on which the Sagartiidæ, Choriactidæ and Paractidæ are separated from the Metridiidæ, Chondractiniidæ and Actinoscyphiidæ.

Flosmaris, therefore, is the type-genus of another new family, the Flosmarinidæ, which will be characterised by the main features of its type-genus.

For the present, however, I do not wish to insist too much on this family Flosmarinidæ, but I simply establish it tentatively pending further knowledge. I do this because the type-genus is as yet known only from one single specimen, and until more come to hand one can never be sure that there is not something abnormal about a solitary individual. However, I am inclined to think it is really genuine and not abnormal; it is a beautifully regular and well-developed specimen, and well preserved. It might be suggested that it is an overgrown Phellia which has doubled its organs. This is possible of course, but nevertheless it would be curious, because some genuine Phellias as large as Flosmaris still retain only six pairs of macrocnemes, the other mesenteries remaining small. The point cannot be finally settled as yet.

The remaining question is, whether any other combinations hitherto unrecorded are likely to come to hand beside the Flosmarinidæ combination. Of course no one knows, but it does not seem at all unlikely that some of them at any rate will do so. It would not be surprising, for instance, if forms bearing the same relation to the Diadumenidæ and Marsupiferidæ as Flosmaris does to the Phelliidæ (i.e. with the same combination of acontia and cinclides as those families, but with twelve or more instead of only six pairs of macrocnemes) should be recorded sooner or later. With regard to the other possible combinations—all three involving forms with a mesoglœal sphincter and probably a pedal disc but with cinclides and no acontia—it seems much less likely, from one's general ideas about the group, that these exist, but of course they may do so.[1] Some forms about which little is known, and which I have not assigned to a definite position in this paper, are Stephanactis and Amphianthus. The former as described by Hertwig has cinclides but no acontia, and may possibly represent one of the other combinations. I have some specimens of Stephanactis which I may be able to describe sooner or later in connection with this.

With regard to Isophellia, it is hardly yet possible to form a final opinion. I. sabulosa is described by Carlgren (1900, p. 72) from a single specimen, and this specimen seems intermediate between Phellia and Flosmaris in that it has three extra couples of macrocnemes in addition to the six primary pairs. And even some at least of the remaining weaker mesenteries of the second cycle seem to have some of the characters of macrocnemes. Only further material will make us certain whether it is an unusual and exceptional member of the Phelliidæ or a not quite fully developed example of the Flosmarinidæ. I think the latter is quite possible. For the present, therefore, I have placed it with a query in the Phelliidæ, and it can easily be removed if the family Flosmarinidæ makes good, and if it really belongs there.

A further note with regard to the position of Flosmaris and of any further possible combinations in my evolutionary scheme will be found on p. 507.

VII. Suggestions concerning the Evolutionary History of the Forms with Acontia or a Mesoglœal Sphincter or Both.

I should like to make it clear that the nine families outlined above (and defined later) are not dependent for their validity upon the acceptance or otherwise of the following suggestions. They seem to represent the only natural grouping in any case, but the evolutionary scheme which will be developed below renders the arrangement more intelligible, and appears to fit the facts of the case.

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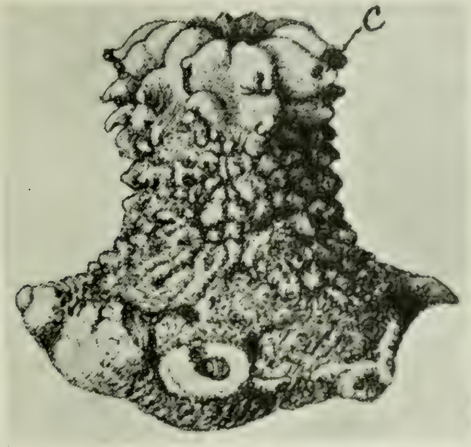
[1] Halcampa, of course, has a mesoglœal sphincter and cinclides but no acontia, but this comes rather outside the scope of the present paper, and is probably fairly near the ancestor of all the forms here discussed. See evolutionary arguments.

In endeavouring to work out some idea of the way in which these forms evolved from a common ancestor, the first step is no doubt to try to decide fairly which of the nine families contains the most primitive forms. In order to do this it is necessary to find out what characters are primitive, and perhaps the best way to do this would be to compare some fairly primitive form with an advanced one. Two convenient ones would be Halcampa chrysanthellum and Catadiomene atrostoma. I select these two simply because they illustrate my meaning well, but others might be substituted for them with slight alterations. I imagine it will be generally admitted that Halcampa is a fairly primitive form. It is more advanced, of course, than Protanthea, Edwardsia, etc., but still is near enough to the ancestral form of the majority of the Actiniina to serve our purpose. I do not wish to go too far back, since I am discussing the evolution of one portion of the Actiniina, not of the whole of the Actiniaria. What claim has H. chrysanthellum to primitiveness? It is small, and its body-wall is delicate and perforated by pores. It is not very different from a certain stage of larval development through which most Actiniina pass, and which may be called the Halcampa stage. Its mesenteries are few in number and its sphincter is weak. The longitudinal musculature of the tentacles has not sunk into the mesogl&oelig;a. Its base is a physa, and it has developed no pedal disc or differentiated basilar and parieto-basilar muscles. On the other hand, what claims has C. atrostoma as an advanced form? It is a large animal with a well-defined pedal disc, and in correlation with this a sharp differentiation of basilar and parieto-basilar muscles. It seems to have taken to deep-water life, and, as in many other deep-water animals, it has developed a thick body-wall devoid of pores, and has lost mobility. The body-wall has also a certain development of knobs and crests (not as conspicuous here as in some other cases, cf. Text-fig. 24, which shows knobs and crests in Actinauge, and Text-fig. 25, which shows knobs in Chondrodactis), and at the base of every tentacle, on the outer side, is a solid mesoglœal swelling. These are all points which seem to me to indicate an advanced stage of evolution. What is the reason for them? The thick body-wall, the knobs and crests and tentacular swellings are found in a number of anemones which appear to be advanced forms. They may be partly protective, but we need not lay too much stress on that aspect of them, because they do not seem efficient enough to act as any great deterrent to such an enemy as a determined fish, for instance. There are other deep-water anemones, and successful ones to all seeming, which have surprisingly delicate body-walls (e. g. Bolocera longicornis). On the other hand, the spreading of an anemone to a deep-water habitat is probably a change towards a slower

TEXT-FIG. 24.



Actinauge richardi. *c.* Capitulum.

metabolism. We know that there are curiosities of respiration, for instance, in the molluscs Pleurotomaria and Lepeta, which apparently have slow metabolism. The marked uniformity of low temperature in deep water may be connected with low metabolism and respiratory difficulties. If the ratio of anabolism to katabolism changes value, there is energy to spare which may be used up in decoration, and I am inclined to regard the knobs, etc., of deep-water anemones in this light. They do not seem to be features which can have any great importance to the animal in its life, save that increased thickness of body-wall means a certain loss of mobility, and is therefore detrimental rather than otherwise. It would not be altogether out of place to compare the basal swellings, crests, etc., of these anemones (especially of Actinauge) with the knobs and spines developed by such groups as Dinosaurs and Ammonites in the late phases of their evolution. It is not impossible that they indicate a last phase in the evolution of these anemones, especially if we consider that the deep sea is a refuge and that the forms possessing them are becoming somewhat less active and are advanced in other ways. Nevertheless, I suggest it with reserve as a mere possibility.

To get back to C. atrostoma, it has other characters giving it claim to an advanced condition. The longitudinal musculature of the tentacles and radial musculature of the oral disc are entirely embedded in the mesoglœa, and this is obviously an advance on the condition in which that musculature is ectodermal. If we take all the cases of Actiniine anemones in which this embedded musculature occurs, whether they have mesoglœal sphincters or not, we find that they are all forms with *numerous perfect mesenteries*,[1] i. e. it never occurs in forms in which the number of mesenteries perfect is limited to six pairs, or in which the division into macrocnemes and microcnemes is found. This seems to suggest that, since forms with the embedded tentacular musculature must at all events be moderately advanced, the condition with numerous perfect mesenteries is also an advance on the state where few are perfect. C. atrostoma has a very large number of mesenteries, and not only are many of them perfect, but the primaries are sterile. Lastly, the sphincter is well developed. If we assume for the moment that the characters I have enumerated for Halcampa are primitive, and those for Catadiomene advanced, I believe there is a reasonable way in which we can account for their being so. It seems to me to be a tendency of Actiniaria in general, broadly speaking, to specialise in size of the individual, and perhaps more especially in diameter. The small size and worm-like form of Halcampa are not adapted to the possession of many mesenteries. It seems reasonable to suppose that, starting from small forms with few mesenteries, if we get increase in size (especially diameter) we may expect a correlated multiplication of organs, i.e. increase in the number of mesenteries. Again, if the mesenteries were at first few in number they would have to do all the work, and primitively they were no doubt all alike, division of labour coming later on with increase in number. This being so we should expect that each mesentery would bear a strong retractor muscle, a gonad, and a filament. This is actually the case in certain fairly

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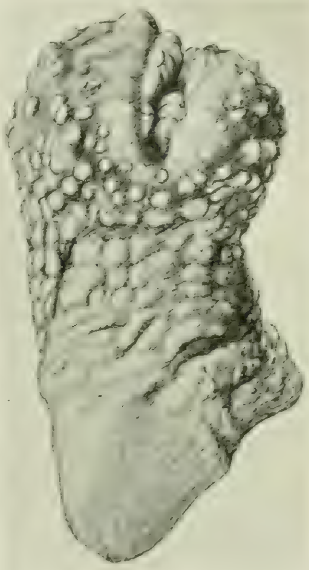
[1] I am not aware of any exception to this among the forms where the musculature is truly and entirely embedded. There are cases in which the ectodermal musculature tends to be partly embedded at its base (meso-ectodermal) in which the rule does not apply, but these are rather different.

Text-fig. 25.



Chondrodactis duplicata.

primitive forms (e. g. some species of Halcampoides). New mesenteries when they appeared would at first be narrow and rudimentary (perhaps until there was more room for them); and we find that our Halcampa represents this stage, having six pairs of large macrocnemes to do the work and a few very small microcnemes which take no share. As evolution progressed the new small mesenteries would tend to increase in size and importance, and take over some of the work from the primaries. The primaries would need less powerful musculature, since they would have the aid of the secondaries in the work of retraction, and their muscles would tend to become less individually specialised. In Halcampa we find, therefore, excessively strong muscles on the few macrocnemes, in Catadiomene less strong diffuse ones on all the older mesenteries. With the increase in size and number of the secondary mesenteries, the growth of the primaries would tend to become fairly complete before the animal approached the adult condition. The production of gonads is usually from tissue which has retained a more or less embryonic condition, and would therefore be more likely to occur in the newer and still growing mesenteries than in the older perfect ones. This should not be taken to imply any suggestion that no increase in size takes place after the appearance of gonads, but rather that the appearance of gonads on younger mesenteries, broadly speaking and with some exceptions, seems to indicate that the process of differentiation has passed its climax in the oldest mesenteries.

All this assumption of importance by the secondary mesenteries would mean that the sharp distinction which is present in forms where they are rudimentary into the original large macrocnemes and a number of small microcnemes would be gradually lost. There would then be several cycles of well-developed mesenteries grading off into each other, and all the older ones would bear retractors and filaments. The tendency would be for the primaries to become sterile. At first the primary mesenteries, which represent the original macrocnemes, would still so far predominate over the others that they alone would be perfect. But in the end the secondary and tertiary mesenteries would join the actinopharynx also, and a form with numerous perfect mesenteries would be attained.

The increase in size of the mesoglœal sphincter is an obvious advance, in working from a small form to a larger one. I will refer in more detail to the evolution of a pedal disc shortly.

Other anemones, generally looked upon as fairly primitive, present features broadly similar to those of Halcampa. Opinion will naturally differ as to which are primitive, but it seems safe to suppose that Gonactinia, Protanthea and probably Oractis form one primitive group, and the Edwardsias another. Halcampa and Halcampoides represent a step onwards, and then there are the probably rather more advanced but still fairly primitive genera Pentactinia, Peachia, Haloclava and Eloactis. There is also the genus Harenactis—rather an unusual one.[1]

The Gonactinia-Protanthea group (probably including Oractis) must be considered apart from the others as perhaps representing survivals of the earliest anemones of all, which have developed in some directions and remained primitive in others. Their primitive traits are their small size, weak and somewhat generalised musculature (surviving even in the body-wall ectoderm), and the presence of only eight perfect mesenteries corresponding to the macrocnemes of Edwardsia. They have an attachment disc but no sharply-marked basilar muscles.

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[1] While referring to the group of anemones mentioned here, it would perhaps be as well to mention that the group "Ilyanthidæ" to which they belong will need splitting up if the principles set forth in this paper be accepted. I am not yet in a position to suggest any detailed classification of the Ilyanthidæ; but I should like to mention one or two points about them. In the first place it seems that Halcampa and Halcampoides, and possibly others with them, will form one natural group, the Halcampidæ. Secondly, Ilyanthus is a genus containing two forms which are widely different from each other, and each of which needs separate consideration from the rest of the "Ilyanthidæ." It is not impossible that I. mitchelli and I. parthenopeus may even be allocated to different families. With regard to the rest of the forms under discussion, Peachia and others, judgment must be reserved at present. The ground for the splitting will *not*, however, be solely the character of the sphincter muscle, but the sum of the main characters.

The other genera enumerated really concern us more here, as they are nearer to the forms whose evolution we are trying to elucidate. They have in common a more or less vermiform shape; in the broadest of them length predominates over breadth quite distinctly (even Peachia may be very long and narrow when alive), and in the narrowest their length is very many times their diameter. They typically have their base developed as a physa. The number of mesenteries is comparatively small, and most often either all are macrocnemes or else there is a distinction into macro- and microcnemes, although in some forms one would have to speak of a grading between larger and smaller mesenteries. The macrocnemes bear strong retractors which are generally more or less circumscribed and frequently reniform in outline in section. Among these forms cinclides occur fairly often. There is among them a complete absence of those characteristics which I have mentioned as typical of certain advanced forms—there are no well-marked sphincters, no large number of mesenteries grading off into each other, no sterility of the primary mesenteries taken as a whole, no well-marked foot-discs with basilar muscles, no very thick body-walls with tubercles and crests and tentacular swellings like those of Actinauge, etc., and no complete sinking of tentacular musculature into the mesoglœa. Instances might be multiplied, but the general tendencies of evolution which I have indicated above are quite widely borne out by the generic series included in the nine families which I have outlined above. There are, needless to say, a good many exceptions here as everywhere else in the animal kingdom, but they hardly invalidate the main scheme. Further notes on these exceptions will be found on p. 508.

Before leaving this section of the subject, a brief study of the evolution of retractor muscles and of definite pedal discs with basilar muscles, in correlation with one another and with the life of the animal, offers attractions. I am indebted to Prof. G. C. Bourne for a number of detailed suggestions connected with this problem. I have mentioned above that the anemones cited as being more or less primitive have all, except in the Gonactinia-group, strong retractors which are usually more or less circumscribed (see Text-fig. 11). They also have typically rounded aboral ends, and their general habit is to live more or less buried in sand or mud, or in tube-like holes. The forms among them with which I am personally acquainted, or of the habits of which I have knowledge from other sources, have the power of contracting with great violence and rapidity. An Edwardsia or a Halcampa can disappear below the sand in the twinkling of an eye. The powerful retractors come in here as shorteners of the long body in cases of emergency. Here we have an interesting case of adaption to a burrowing life or a life in holes. In the Phellias (which have the strong circumscribed retractors) described by Bourne and Haddon and others it is possible to trace a transition from burrowing forms with a real physa to forms with an adherent but physa-like base which anchor on to pebbles, shingle, etc. In such cases the action of the powerful retractors cups the physa-like adherent base and makes a sucker of it. This shows the first step towards the development of a true adherent base. From this point onwards there is no difficulty about imagining the evolution of a more definite and permanent pedal disc, and in correlation with it a change in the form of the lower ends of the mesenteries for insertion into it, and a differentiation of definite basilar and parieto-basilar muscles. But given this broad adherent base, a stronger body-wall, and a well-developed sphincter, the animal does not need to contract so violently. It has presumably left its sandy quarters and is attached to a rock or some other similar substratum, and one might expect its retractors to degenerate and become diffuse. This is further borne out if we remember that at the same time as this evolution is progressing the mesenteries are increasing in number, and that therefore as soon as more of them have attained retractors, there will be less need for specially strong individual muscles on the primaries. On these grounds I think it is a fair conclusion that the general tendency is for circumscribed retractors to become diffused as evolution progresses. There are a few forms which retain the circumscribed retractor even though they have largely lost their other primitive characters. In these cases the life and habitat of the animal may be looked to for an explanation. A further note about them will be found on p. 511.

One fact cannot be passed over. It is not invariable for primitive forms to have powerful or circumscribed retractors; in Gonactinia and Protanthea the retractors are very feeble—hardly differentiated. In the first place I think it is probable that the ancestor of the Actiniaria as a whole would have weak retractors and a non-concentrated muscular system generally. It would no doubt possess longitudinal musculature in the ectoderm of the body-wall, which would obviate the need for strong retractors. It would probably be a small, delicate animal whose tissues would not offer much resistance to the forces of contraction. As far as the ectodermal muscle-sheet is concerned it might be compared with Cerianthus, especially as some authors consider a Cerianthus-like form to be primitive. Cerianthus inhabits sand and mud, and has practically no mesenterial musculature, but in substitution a very powerful ectodermal musculature in the body-wall. As Carlgren holds, this feature may be in part retained by Protanthea and Gonactinia, but it is lost in the higher anemones on the whole, with the exception of a number of unrelated forms which retain traces of it. It is not impossible that mode of life will help to account for this retention (or re-development)—in part at any rate. In Aiptasia couchi, for instance, the weakness of the rest of the musculature stands in need of a little aid from ectodermal muscle. The life conditions of Gonactinia and Protanthea do not seem to indicate any special need for strong retractors. Both are small and delicate; they live in fairly shallow water attached to seaweed and other things. Carlgren records Protanthea on Chætopterus-tubes and Gonactinia on Ascidians and dead mussel shells. Gonactinia can creep by means of its tentacles, but can swim mouth foremost and so remove itself from any small disturbance. Oractis, which is probably related, is a small deep-water form. It is therefore possible to regard the Protanthea group as representatives of the most primitive condition retained by any existing forms, and to suppose that the rather more advanced forms with circumscribed retractors have developed these in correlation with mud-burrowing and life in cavities, and perhaps also in correlation with the loss of the ectodermal musculature of the body-wall. If this be the case we must look upon the circumscribed retractors as specialisations in otherwise primitive forms. The further development of lines of evolution starting from these forms, and involving a change to a fixed life and including increase in the number of mesenteries, would mean the loss of these special retractors in most cases and the differentiation of a larger number of diffuse ones.

With the above preface I will indicate as briefly as I can the line of evolution which seems most in accordance with the facts, as regards my nine families. Afterwards possible alternatives will be mentioned.

Following the lines indicated above, it is natural to choose as a starting-point that form which most nearly expresses the characters which have been enumerated as primitive. The group of the nine that comes nearest to this is the Diadumenidæ. The only difficulty here—and I do not personally consider it to be a difficulty—is that acontia are present. But, as has been noted before, acontia are only a variety of mesenterial filament, and if filaments have been acquired by the very simplest anemones, such as Gonactinia, why should it not be supposed that the slight differentiation needed to produce acontia appeared at a fairly early stage in the history of the group? If a form has gone far enough to acquire a mesoglœal sphincter, it has surely gone far enough to acquire acontia, and there is no valid reason why a start should not be made with a form possessing the latter. Mena chilkaea[1] fulfils our requirements almost exactly. It is rather small and delicate. The column is capable of considerable elongation, and has a small unmuscular basal disc never strongly adhesive, and capable of assuming a physa-like appearance. The delicate wall is pierced by cinclides. There is a weak mesoglœal sphincter. There are six pairs of macrocnemes with circumscribed retractors, and very few (usually 12) microcnemes, which are almost vestigial. Acontia are present. I think we may assume that this comes very near to the ancestral form we are looking for. It is a species, certainly, the simplicity of which may be due to retrogression, in correlation with its life (see p. 509) in brackish water, but that does not interfere with its giving the necessary clue to the primitive type. It is probably secondarily primitive.

My hypothetical ancestor may therefore be defined as follows:

Body-wall delicate and pierced by cinclides; form more or less elongate; six pairs of fertile macrocnemes with circumscribed retractors and with filaments; microcnemes absent or very few and quite rudimentary; acontia present; sphincter weak, mesoglœal, only just distinctly developed; base a physa.

It should be understood that this form, which I will call for convenience Eosagartia, is only suggested as the probable ancestor of the genera contained in the nine families above enumerated—the genera with mesoglœal sphincters or acontia, or both. It is by no means the ancestor of either the whole of the Actiniina or Actiniaria; it is about on a level with Halcampa, and very likely it shares with Halcampa a rather earlier common ancestor.

In order to make what follows clearer, I propose to give the hypothetical history of the group in narrative form, but of course I have no intention of stating that it *did* happen so, but am merely using that mode of expression as the clearest.

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[1] *Mena* = n. nom. for *Phytocoetes*, pro parte. See p. 522.

Eosagartia lived in the sand, into which it could withdraw very suddenly when necessary by means of its powerful retractor muscles. In correlation with this kind of life it had a long body and a rounded base. The pores in its delicate body-wall helped to allow water to escape from inside it without bursting the wall, when it had to contract quickly. Its acontia were sometimes forced out through these pores by the strong jets of water which spurted out of them.

Some of the descendants of Eosagartia developed certain features which made them unlike their original parents. They acquired a basal disc, by which they attached themselves to solid objects, and the number of their microcnemes increased, and some of them began to assume in some degree the importance of macrocnemes. How far this particular branch of descendants diverged from their ancestral condition we do not know, but in the end they took to living in brackish water in India, very often returning to a life buried in mud (Pl. 22, fig. 13,; Text-fig. 28). This kind of life involved loss of some of their specialisations, and they became somewhat simplified again, so that in the end some of them almost went back to the condition of Eosagartia. Most of them even lost their mesoglœal sphincter. The survivors of this branch, therefore, retain their primitive features in the main to this day. They are the *Diadumenidæ*.

Another group of those descendants of Eosagartia, in which the microcnemes had begun to increase in number, began to develop a protective cuticle on the main part of their body. This meant that the wall lost delicacy, and was no longer a suitable place for pores, and these were lost also. About this time some of them began to attach themselves temporarily to bits of shingle by making a sucker out of their base—they pulled it inwards with their strong retractors. This led in some cases to the development of a more or less definite pedal disc. Another thing that happened was that in some of them the acontia began to degenerate, and in the forms now surviving it is possible to find, in this group, forms provided with good acontia and forms in which they are rudimentary. Then, again, the microcnemes tended to grow filaments and acontia. But although many forms which followed this line of evolution still survive, they have never lost their six pairs of great macrocnemes bearing gonads and strong retractors as well as filaments, and reaching the actinopharynx, and the microcnemes are still more or less insignificant, and clearly marked off from the others. They have retained, too, as a rule, rather long bodies, and the power of introverting the disc and tentacles and the delicate neck or *capitulum* which bears them within the tougher cuticled *scapus* or middle part of the body. Even part of the scapus can be pulled in as well. Many of them have developed a better sphincter than Eosagartia had, and it has often divided into two parts—one in the scapus and one in the capitulum. This group of forms constitutes the *Phelliidæ.*

Thirdly, some of the species related to the Phelliidæ went a step further. They had already lost their cinclides. They did not lose the primitive distinction of the mesenteries into macrocnemes and microcnemes, but their acontia, from being rudimentary, disappeared entirely. They developed a rather more definite kind of adherent base. As regards the body-wall, they retained the division into a main scapus with cuticle, or with some sort of incrustation, and a submarginal non-cuticled capitulum. They survive in this condition as the *Marsupiferidæ.* One of them has developed curious brood-pouches in the female.

It is now necessary to go back to the beginning and follow the further evolution, in quite another direction, of some others of those descendants of Eosagartia in which the number of microcnemes had begun to increase. These, instead of losing the delicacy of the wall, the pores, and the acontia, developed more as regards the mesenteries and the base. They adopted an adherent life, and this was followed by the development of a well-marked base. The microcnemes increased in importance, and in the end they developed so many structures that they approached the condition of the macrocnemes, and finally the distinction between the two kinds was lost. The secondary mesenteries mostly acquired filaments and retractors, and often the work of producing gonads fell upon them, because the primaries had passed the climax of their growth rather early on. Although these things occurred, the primary mesenteries, which had originally been the macrocnemes, still remained predominant in one respect—they alone joined the actinopharynx, the others remaining imperfect. Here, then, a stage is reached where the distinction into macrocnemes and microcnemes is lost, and the mesenteries are arranged in graded cycles, but still only six pairs of mesenteries—the primaries—are perfect. As the muscular mesenteries increased in number and the body-wall often became rather less flimsy, and as the basal disc became better developed and the sphincter stronger, there was less need for the retractors of the primary mesenteries (the old macrocnemes) to be exceptionally powerful, and they degenerated in most cases, and became diffuse like those of the secondary or imperfect mesenteries (the original microcnemes). From this point onwards some forms began to diverge in one direction, some in another, but a number of forms got no further, and represent this stage at the present day as the *Metridiidæ*.

There are one or two of the *Metridiidæ* which either took to some kind of life which meant retrogression, or else did not quite keep up with the others in all respects; and these, though they have the typical mesentery character, have the mesoglœal sphincter either very weak and rudimentary or quite absent. Some of them have even kept or returned to the flimsy body-wall with a certain amount of muscle in its ectoderm, and a poorly developed base, which sometimes lets go its hold on the rock sand and, as the animal lies loosely on its side, is inflated so that it is half like a physa. This applies to Aiptasia couchi, for instance.

From this point onwards there are two distinct branches of further divergence to follow. Some of the forms near the present-day Metridians went in one direction, some in another.

Let us first follow those in which, to the very end, the six pairs of primary mesenteries so far predominated over the others that they alone were perfect,[1] although the other mesenteries ran them close and bore filaments and retractors (except for the very young ones) and generally gonads as well. As regards other things, however, many forms began to follow a line similar to the one already traced in the Phelliidæ. The body-wall became generally tougher and thicker and lost its cinclides, while the lower part of it in many cases developed a cuticle, so that the body was in some degree divided into scapus and capitulum. But things went further than this, because some forms retired into deep or fairly deep water, possibly for refuge, their metabolism became slower, and they often developed not only a thick cartilaginous body-wall, but also used up spare energy by ornamenting it with knobs and crests, the crests sometimes breaking up into more knobs. In some cases the tentacles even developed great solid swellings or horns at their bases. The sphincter still remained strong, but the acontia began to degenerate, and among these forms, as among the Phelliidæ, forms may be found in which they are still well developed and others in which they are quite rudimentary. The genera which have evolved no further than so far indicated are the *Chondractiniidæ*.

Certain animals related to the earlier Chondractiniids not only lost their cinclides, but also all trace of acontia. They retired to deep water and their bodywall in some cases became remarkably ponderous—so thick that one would think the animal could hardly move (see Text-fig. 31). In these cases the sphincter degenerated and became less powerful, and sometimes even the tentacles were reduced, and they developed swellings of mesoglœa also. Some other animals related to the early Chondractiniids also lost their acontia as well as their cinclides, but, probably in correlation with life on shore, sometimes in deep cracks, they retained part of the primitive delicacy of tissues and some other primitive traits (see p. 511). But it is convenient to include them with the deep-water forms in one family, as the two sets have much in common. This family is the *Actinoscyphiidæ.*

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[1] Except in a few cases where a very few other mesenteries attain perfection.

In order to understand the last part of this evolutionary history, it is necessary to make a start once more from the stage represented by the Metridiidæ, and to follow the development of those forms which descended from that stage, but did not go in exactly the same direction as the Chondractiniids and Actinoscyphids.

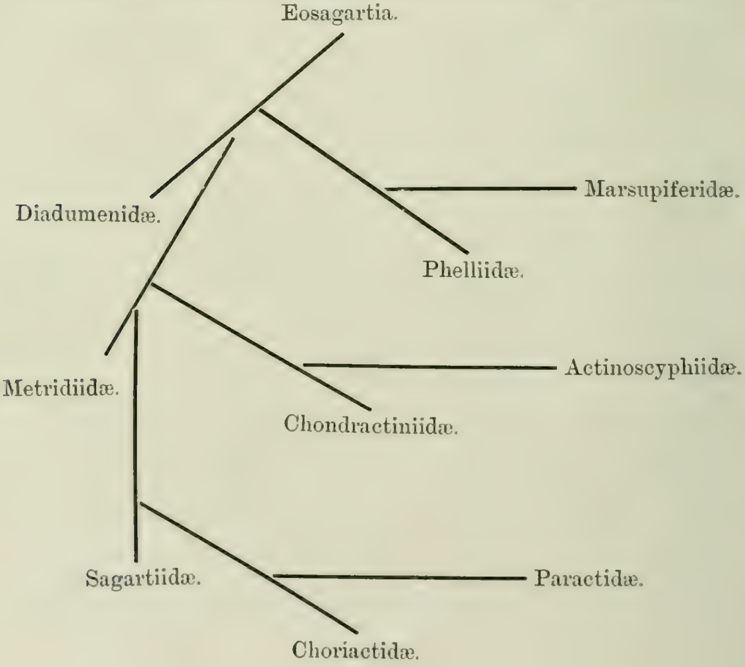
From a form near Aiptasia, in which, although the distinction between macrocnemes and microcnemes had been lost, the primaries still retained their fertility (others sharing it), a number of forms went a step further. The primary mesenteries lost even such predominance as was given them by the fact that they alone were perfect, and the first cycle of secondary mesenteries, and even the second cycle of secondaries (i.e. the tertiaries, counting the cycles another way), became perfect also. At first the acontia, the cinclides and the fertility of the primary mesenteries were retained, and the present-day forms representing this phase of evolution are the true *Sagartiidæ* in the strict sense, i.e. the genera Sagartia, Cereus, and Artemidactis. They have the mesoglœal sphincter well developed.

Some relatives of the Sagartiidæ then began to follow a path similar in some respects to those already outlined as having been trodden by the Phelliidæ and Marsupiferidæ on the one hand, and the Chondractiniidæ and Actinoscyphiidæ on the other. Some forms retained, others lost the primitive fertility of the primary mesenteries. But the body-wall sometimes became thickened, and in all the cinclides were lost. There is only one described genus which definitely represents this particular stage, and that is Choriactis. It has many perfect mesenteries and thick body-wall without cinclides, but acontia are still present and the mesoglœal sphincter is well-developed. This is the type-genus of the family *Choriactidæ.*

The final step is that by which the forms with numerous perfect mesenteries, and with no trace of distinction of the mesenteries into macrocnemes and microcnemes, lost their acontia as well as their cinclides. In the majority of them the longitudinal musculature of the tentacles and radial musculature of the oral disc, which were primitively ectodermal, sank entirely into the mesoglœa, and sometimes even became rather reduced. The forms representing this final stage are the *Paractidæ* in the strict sense, as defined on p. 548. Some of the genera still retain one or two primitive features, but apart from the other advanced tendencies, a number of them exhibit thick body-walls which may develop knobs or crests, and the tentacles may show basal swellings of mesoglœa similar to those mentioned in connection with other families. In the Paractids the mesoglœal sphincter is well developed.

The above statement gives in general outline an evolutionary history which seems to offer a reasonable explanation of the varied structure exhibited by the group.

It might be graphically represented in some such way as this :



The figure shows that the line of evolution from Eosagartia to the Paractidæ is a fairly continuous one, affecting mesenteries first, and in the end involving loss of first cinclides and then acontia, the former in correlation chiefly with a change in the body-wall. Branching off from this main line are two others, running *parallel* with the later part of the first line, and both similarly involving loss of cinclides and finally of acontia. This evolutionary hypothesis explains the continuous evolution of the mesenteries, starting from a primitive condition with a small number of microcnemes. It assumes, of course, the loss of first cinclides and then acontia by three different series of forms independently; but I submit that it is easier to understand their disappearance in three different cases than it would be to understand their independent acquisition by three different sets of animals, which latter would have to be assumed if we started from some other point. The loss of such pores as cinclides is liable to happen to any number of animals progressing on the whole towards less delicacy of body-wall. I do not know whether one could find a reason why useful structures like acontia should disappear, but at all events there is no definite reason why they should not. It is possible that they were an experiment which did not prove so great an advantage as it might have done, and was dropped. Loss of activity and slower metabolism may help to account for the degeneration of such organs in deep-water forms (cf. reduction of gill-respiration in Pleurotomaria), and if these forms had not a sufficiently contractile body how could they expel the acontia? They are forced out, as far as we know, by currents of water escaping from the contracting body. In the case of shore forms and others to which this would not apply the former idea would have to be resorted to. I do not wish to push it too far or to over-emphasise it, but the disappearance of acontia does not seem to me to be a great difficulty, and there may be unknown causes that could lead to it.

It would be advisable now to consider whether there are any alternative ways of deriving these nine families from ancestral forms, and how far any such are valuable. Opinions will naturally differ. The possibility of starting at the Paractidæ and working towards the other groups hardly needs examining, for surely no one would take a form with all the specialisations of Catadiomene (for instance) as primitive (see p. 488). Again, to start from Actinoscyphia or Marsupifer would be to work from forms with either thick walls and bulbous tentacles or with cuticle, and definite or well-developed pedal discs with basilar muscles, towards more delicate creatures with weaker base. It would assume independent acquisition in three cases of first acontia and *then* cinclides, although, as I have shown above, the presence of cinclides is not dependent on that of acontia. Such a scheme would end up near Halcampa, and is, I take it, out of the question.

The only reasonable alternative seems to be to start with Eosagartia as before, but to arrive at the Paractids eventually in a different way. To do this one might take the main line (involving loss of cinclides and then acontia in this case) through forms related to Diadumenidæ—Phelliidæ—Marsupiferidæ—Actinoscyphiidæ—Paractidæ, and assume on three independent lines the changes from a condition with a few to the eventual state where many mesenteries are perfect and there is no longer any distinction between macro- and microcnemes. The two secondary lines expressing this mesentery evolution would be parallel to each other and to the later part of the main line, and would be, respectively, the lines passing through relatives of—

(a) Diadumenidæ—Metridiidæ—Sagartiidæ, and

(b) Phelliidæ—Chondractiniidæ—Choriactidæ.

That the whole series of changes from the macrocneme condition to the state with numerous perfect mesenteries and no distinction into macrocnemes and microcnemes should take place on three independent lines, seems to me much less likely than that cinclides and acontia should be lost on three independent lines. It would be by no means impossible, and even seems to have taken place in some of the other Actiniaria not under immediate discussion, but it would be the less safe of the two assumptions in this case. Again, if this suggested alternative scheme were adopted, it would not work out by means of so neat a series of graduated changes.

After studying the question, therefore, from many points of view and for a considerable time, I have not accepted any of the alternative possibilities, but have concluded that the scheme described in detail above is the most natural and probable. But even if the explanation is not accepted, the classification of the group into nine clearly-defined families will remain unaffected. Of course, if it should be considered that the nine families evolved separately from different ancestors a different explanation would have to be found. But that seems highly improbable, and since the various forms grow very easily out of one another (or rather out of one another's once-existent relatives), running back to an early Halcampa-like form with acontia, there seems no reason why in this case the mesoglœal sphincter should not denote common origin.

Before leaving the question of evolution, I should like to indicate the position in my scheme of Flosmaris (see pp. 441 and 484) and of any new anemones which may be discovered, representing other combinations than those here dealt with (see p. 485).

It is quite possible that Flosmaris might be evolved in either of two ways: it might be derived direct from a Phellia-like ancestor by increase in the number of macrocnemes, or it might be derived, as I suppose others to have been, from an Eosagartia-like form viâ an ancestor, hitherto unrecorded, in which the number of macrocnemes had increased instead of remaining six pairs only, but which had retained cinclides as well as acontia. The loss of the cinclides and development of a different body-wall would lead thence to Flosmaris. I do not wish at present to definitely prefer one of these alternatives until other forms are known. The first one seems the more obvious, but I think the other one is equally tenable, and if the intermediate ancestor mentioned were discovered—

it would represent one of the possible combinations—it might prove preferable. Although Flosmaris is externally like Phellia by possessing a scapus and capitulum, I do not think this necessarily means that Phellia is its ancestor; parallel developments are sometimes striking in anemones, and other forms have scapus and capitulum which could not be connected with Phellia.

I will not make any definite suggestions with regard to the evolution of the other possible combinations referred to on p. 485, which may come to hand, until they do so; but I may say that I have worked out the evolutionary scheme and the tree on p. 504 in such a way that new forms can be filled in without much difficulty.

VIII. Notes on Exceptional Genera.

There are a few genera presenting characteristics which make them exceptional in one or more respects from the general tendencies of the group as outlined in the foregoing evolutionary history. The existence of exceptions in no way disproves the general rule. In dealing with living forms it is impossible to divide everything up into absolutely water-tight compartments on a mechanical basis. The exceptions cited may be understood as either forms in which retrogression has taken place in correlation with mode of life, or in other cases as forms which have retained one or two primitive characteristics whilst losing others.

(1) Forms with no Sphincter.

The genera possessing acontia but no mesoglœal sphincter are Diadumene, Phytocœtes, Pelocœtes, Halcampactis, Bartholomea and Aiptasiomorpha. It will be noticed that all these forms occur in the three families which, according to my interpretation, are the most primitive of those here dealt with—i.e. the Diadumenidæ, Metridiidæ and Phelliidæ. After this the mesoglœal sphincter becomes fixed.

Diadumene, Phytocœtes, and Pelocœtes (Pl. 22, figs.

12, 13, Text-fig. 28).—These three forms live together with Mena in brackish water in India. A study of the excellent account of them given by Annandale (**4**, **5**) will remove all doubt as to their being related to each other and derived from a common ancestor. Mena has the mesoglœal sphincter weakly developed; the other three agree in all other fundamental respects with it, and could on no account be separated from it, except of course generically, the generic differences being very marked. They may never have acquired the sphincter, but I think perhaps it is more likely that they have lost it. Except Diadumene, they are burrowing and sponge- and alga-inhabiting forms with long bodies, and the retention by all of them, even Diadumene, of strong retractors usually circumscribed does away with the need for a strong sphincter. The adaptation to life in brackish water, with a return very often to burrowing, might easily account for a retrogression or secondary simplification involving loss of the sphincter, or a checking of its development (it is hardly efficient even where present) and a general return to a fairly primitive condition.

Halcampactis.—This again is a form with strong retractors which may have lost or never acquired the sphincter. We are not even perfectly certain that it does not possess a very weak one. Farquhar states that it has no "well-defined" circular muscle, but, as Bourne has suggested (**10**, p. 67), a very small one may be present. In any case it agrees with some of the Phelliidæ in all other important respects, and one cannot doubt, on the evidence of the sum of the characters, that it is related to them. It is nearer Phellia than Halcampa, because it has acontia and no cinclides, whereas Halcampa has cinclides and no acontia.

Aiptasiomorpha and Bartholomea.—A number of species have been described under the names Aiptasia and Aiptasioides, on the assumption that the type-species of Aiptasia (A. couchi) was devoid of a sphincter. The discovery of a weak mesoglœal sphincter (see p. 438) in A. couchi, necessitates a certain readjustment of names, which will be dealt with later on (p. 531). As a result there are now four genera—Aiptasia and Heteractis, in which a weak mesoglœal sphincter is present, and Aiptasiomorpha and Bartholomea, in which it is absent. The former genera contain couchi, prima and pallida, the latter tagetes, annulata and others. I take it that no one would suggest that couchi, prima, etc., are not closely related to tagetes, etc.; in all important features save the sphincter the two sets resemble one another. The mesoglœal sphincter is very weak in those which have it, but they provide a link between those without it and those in which it is strong. It looks as if tagetes, etc., had lost a muscle which was getting weak in couchi, etc. (or else as if the sphincter-development had been retarded while other organs evolved). The lax habit of A. couchi and its long non-retractile tentacles must be connected in some way with its mode of life, and would naturally be conducive to degeneration of the musculature. If A. mutabilis has, indeed, as stated by Simon, a diffuse endodermal sphincter, it must have been secondarily acquired to replace the lost mesoglœal one in connection with some new need for additional musculature (see, however, p. 467).

In consideration of the details collected here, it may be fairly claimed that all the forms enumerated as possessing acontia but no mesoglœal sphincter are quite clearly related to other acontiated forms, but have lost the sphincter (or more improbably never acquired it) in correlation with mode of life or something similar. I venture to suggest even that it is not impossible that in some of these forms, at any rate, there is in fact a very weak mesoglœal sphincter. Very feeble sphincters are easily overlooked and require good material for their detection.

(2) Forms Retaining Certain Primitive Characters.

I do not suggest that enough is known about the habits of the animals that will be mentioned to fully account for the structural correlations which make them exceptional to the general tendencies outlined in my evolutionary scheme. But a detailed study of their life and conditions might well reveal an adequate reason in each case.

Paraphellia and Isoparactis (Text-figs. 29, 30) have retained circumscribed retractors on the primary mesenteries (not on the others)—a characteristic which their relatives have lost. Paraphellia generally lives on a sandy bottom, and it may need the powerful retractors to help the sphincter to withdraw the tentacular crown very suddenly to avoid its being bitten off by hunting fish in search of such things as molluscs emerging foot-foremost from the sand. This or some similar reason would account for retention or re-development of the circumscribed muscles. Isoparactis lives under stones and in dark places. Another anemone which has to some extent retained circumscription of retractors is Hormosoma, an Antarctic form, the life conditions of which I do not know. The retractors in this case are less powerful and definitely circumscribed than in the other two.

Stomphia, Isoparactis, Paranthus, etc.—The tendency towards the formation of a thick or cartilaginous or cuticled body-wall is not universal though general, and sometimes a thin or comparatively thin one persists. Stomphia and Isoparactis, whilst possessing a firm and well-developed mesoglœa, vary from moderately thick to quite thin walls. Paranthus is another case in point. Paranthus and Isoparactis are shore forms; Stomphia lives in moderate depths. It is natural, of course, to expect thickening of the wall to be best developed in deep-water forms, which is actually the case, but even here Ophiodiscus is not really thick-walled. The exact thickness varies of course according to how much the body is distended with water, but probably the thickest walls cannot be much altered.

Gonads.—Although the general tendency seems to be towards sterility of the primary mesenteries, a number of forms still produce gonads on the primaries as well as some of the others right to the end of the series. These forms are some Aiptasids, Isoparactis, Paranthus in some cases, the true Sagartiidæ (Sagartia, Cereus and Artemidactis), and some of the Paractid genera. The Aiptasids and Sagartids are still not so far removed as many forms from the original state of the group, and we can understand the retention in such cases. I can offer no explanation of it in the Paractid case. Isoparactis and Paranthus are curious forms, exceptional in most things. Further reference will be made to them in enumerating the genera.

IX. Discussion of Generic Characters.

(1) Form of Base.—When a pedal disc is present it may vary considerably in one and the same species, as regards its form, according to environment, and is therefore not valuable for a specific or generic character unless in any given case it exhibits certain characteristics invariably. Verrill states quite clearly, for instance (**93**), that in Actinostola callosa, Hormathia longicornis and H. nodosa the base takes the form of a hollow mud-clasping cup when the animal is living in a muddy situation, but that when it is living on a hard, stony bottom the base takes the form of a flat attachment-surface. In the genus Actinoscyphia the base may form a mud-cup, or it may be adherent or clasp the spine of some animal and so become long and slit-like. In Actinauge richardi stages are found from a broad, flat adhesive disc to a deep cup-like hollow with only a small circular opening, according to habitat. It is probable also that anemones which live habitually on such supports as Gorgonians and have one axis longer than the other would be normal in shape if they grew up under more ordinary conditions, and this one feature is not enough to differentiate them generically from ordinary forms if they agree in all the more important respects.

(2) Body-wall.—Presence or absence of definite verrucæ or acrorhagi and similar structures are obviously useful generic characters. Presence or absence of a cuticle on the scapus may also be valuable. With regard to the presence or absence of "capitular ridges" in some forms, it would seem wise to

discard the use of this character as a generic one. In Hormathia digitata the capitulum may be smooth (Haddon's specimens) or definitely ridged (many of Carlgren's specimens). In two specimens of this species which I have examined one had the ridges much better developed than the other. I have already shown (**80**) how much these ridges vary in Actinauge richardi, in which they may be nearly absent or very prominent. When present they are more or less regularly arranged in some Hormathias, but in Chondrodactis duplicata have no definite arrangement, and their presence in this latter case cannot separate C. duplicata from other species of Chondrodactis without definite ridges, because in other ways the relationship is so clear. In Cymbactis gossei there are "capitular ridges" which are quite prominent but without regularity; in this case the only recorded specimen was strongly contracted, with the upper part of the body introverted, and the ridges seemed to me to be due to the contraction of the thick wall, and would probably disappear on expansion. The species seems to be clearly a Cymbactis in spite of them. Considering the general variability of these ridges, even within one and the same species, it would seem best to discard this somewhat trivial character as a sole mark of difference between two genera.

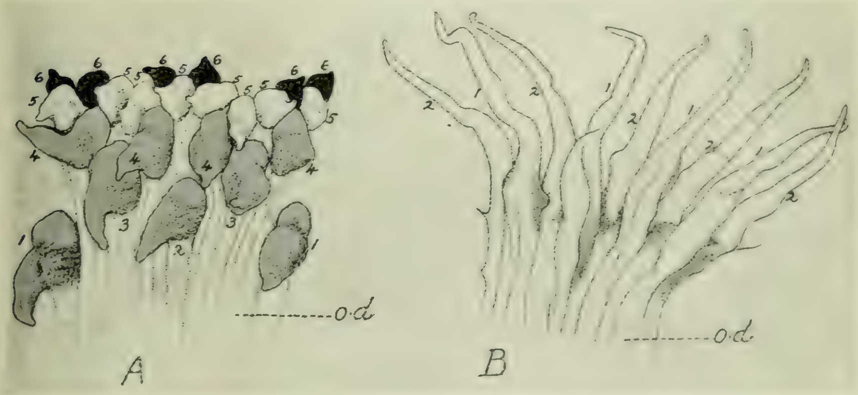
The actual thickness of the body-wall itself is another debatable point. It has seemed a rather useful character sometimes and has actually been used. But recently I have come across cases in which it is difficult to decide whether such-and-such a species should be classed as "thick-walled" or "thin-walled." For instance, some specimens I have of Stomphia churchiæ have a quite moderately tough and thick wall for the size of the animal, but I would not call them exactly "thick-walled" or "thin-walled." Carlgren's figure of Stomphia (Taf. i, fig. 11, 1893) shows quite a thin-walled specimen, so it is evidently variable. Then when I was studying S. churchiæ its whole organisation reminded me very strongly of a form I had previously described as

Cymbactis selaginella (**79**, p. 36), both as regards arrangement and appearance of tentacles and mesenteries and as regards detail, so that I have no hesitation in saying that both ought to be included in one genus. But C. selaginella may be called definitely "thick-walled," though not necessarily so, and evidently here the genus cannot be characterised one way or the other since it must also include S. churchiæ. In Chondrodactis, again, one finds grades of thickness, from moderately thin in part in C. pulchra to very thick in some specimens of C. duplicata. In Actinauge richardi different shades are found. Some specimens have a very thick, firm mesoglœa, quite remarkably solid for the size of the animal, while in others it varies in thickness, till in cases where the body has been distended with water the wall is so very thin that it is semi-transparent and the mesenterial insertions show through it. Since this character is liable to vary and one cannot draw a line between "thin-walled" and "thick-walled" forms, it would probably be the best to discard it also, in most cases, as a sole difference between one genus and another.

(3) Tentacles.—The shape of these organs when they are simple is hardly a useful character for defining genera, because it varies very greatly from time to time in one and the same living individual, and within the limits of one species. The form of the tentacles in a preserved specimen will depend chiefly on the circumstances of the animal's death. I have, for instance, two specimens of Phytocœtes gangeticus preserved in one tube; one of them has the tentacles contracted—short, and comparatively stout and stiff; in the other they are expanded, and are very long and slender and quite flabby.

One character has struck me during the course of my work as being a distinctly valid one for the separation of genera. In Actinoscyphia, Chondrodactis and Polysiphonia, for instance (three genera not closely related to one another)—genera containing large, sometimes very large, species—there are never more than two cycles of tentacles, although the mesenteries are arranged in several cycles in the usual way. It may be urged that the exact number of cycles of tentacles is not much to go by, and in many cases it is not. But in the cases referred to there is a wide oral disc, and the tentacles are all placed in two alternating cycles at the very edge of it, as a rule all of them more or less communicating with the margin of the body by means of their basal swellings, the outer ones only being more definitely marginal than the inner. The appearance is quite different from the usual cycle-arrangement, and there is no marked diversity in size between the outer and inner tentacles. I have attempted to bring out this difference in Text-fig. 26. A represents part of the disc and tentacles of Catadiomene atrostoma (diagrammatic), showing the ordinary arrangement in several cycles—five and part of a sixth in this case. B shows part of disc and tentacles of Actinoscyphia saginata (diagrammatic), with the two alternating cycles only. In A the sixth cycle tentacles are black, the fifth cycle white, the

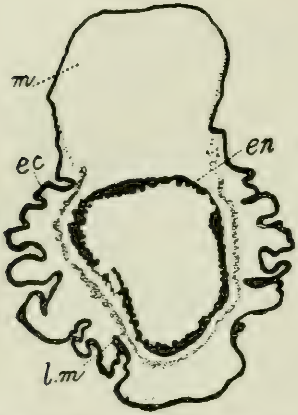
TEXT-FIG. 26.



A. Portion of oral disc and tentacles of Catadiomene atrostoma (diagrammatic). B. Portion of oral disc and tentacles of Actinoscyphia saginata (diagrammatic). *o.d.* Oral disc. Numbers indicate tentacle-cycles.

others grey. In anemones attaining so large a size as many of these, it is quite unusual for the tentacles to constitute only two marginal cycles, while the mesenteries at the same time arrange themselves in several cycles in the usual way. It probably indicates an unusual mode of appearance of the tentacles, and should form the basis of a clear generic character. Of course there will be a stage in the life of any young anemone when only two cycles of tentacles are present, but I am speaking of adult forms, the majority of which attain several cycles.

Text-fig. 27.



Transverse section of tentacle of Catadiomene atrostoma. *l.m.* Longitudinal musculature. *ec.* Ectoderm. *en.* Endoderm. *m.* Mesoglœa.

There are some forms in which some or all of the tentacles bear, on their outer sides at the base, solid thickenings or swellings of the mesoglœa of their aboral walls. Text-fig. 27 shows a transverse section through the base of one of these tentacles. Here the swelling is much less conspicuous than is often the case. The presence or absence of such swellings provides a very simple and useful generic distinction, since it appears to be stable. Forms which possess the swellings, and forms without them, may, it is true, be very closely related in other respects, and even when the swellings are present they may be variable in development, so that in one and the same species they may be very large and conspicuous in one individual, and so small as to be hard to detect at all in another. But they may be detected with care even at their weakest, and it is their *presence,* not their *size,* that constitutes the generic character.

Another interesting development is shown by the tentacles of certain relatives of Aiptasia—Heteractis and Bartholomea (see pp. 531–2). In these genera the tentacles develop curious thickenings, raised above the general surface of the tentacle, and which may take the form of spherical tubercles or of incomplete annuli or spiral bands. These are perfectly definite structures, differing histologically from the rest of the tentacle chiefly in that they are batteries of nematocysts; the ectodermal musculature of the tentacles may also be reduced in them. As far as I can glean from the accounts I have of these species, the presence of the bands on a portion of the tentacle (B. peruviana) or over a greater part of its extent is a constant character. It seems to be quite as worthy of generic rank as the presence of basal mesoglœal swellings, and is a very convenient and easily made-out feature. The genera with and without these curious tentacles are certainly nearly related, but for distinguishing genera one does not need many differences. If it should be shown that the presence of the thickenings is variable and that they are absent in certain individuals that might alter the matter, but even then I am not sure that the potentiality for producing them in one species and the inability to do so in another would not be a sound generic distinction.

(4) Mesenteries.—The distribution of gonads on the mesenteries is of general use in a broad sense as indicating evolutionary tendencies within the whole group, and in some families either one method or another is adopted throughout. But some of the higher families are variable in this respect.

In the families Diadumenidæ, Phelliidæ and Marsupiferidæ, where there is a division of the mesenteries into macrocnemes and microcnemes, the distribution of gonads of course follows and forms part of this distinction, the gonads being borne by the macrocnemes except in exceptional individuals.

In the families where there is no such division into macro- and microcnemes there are two in which one rule is universal. In the Chondractiniidæ the primary mesenteries are sterile, and in the Sagartiidæ (sens. strict.) most or all of them are fertile, as well as others. But in the remaining families the rule is different for different genera. In most cases all the species in a genus adopt exclusively one condition or the other—i.e. either they all have sterile primaries, or they all have them fertile. But there are a few cases of variation even within one genus or species, and it does not seem wise to separate two species from each other on the *sole* ground that one has fertile and the other sterile primaries, although it is very useful to mention the facts of the case in a definition. Miss MacGuire (**66**) records two specimens of Paranthus chromatoderus, one of which had fertile and the other sterile primaries, some of the other mesenteries, in the second case, being fertile; in the first case some secondaries were fertile as well as the primaries. In Bolocera, a genus outside the immediate scope of this paper, some species have fertile and some sterile primaries, and in B. longicornis it seems as if different *individuals* might have fertile or sterile primaries. In any case, of course some of the non-primaries are fertile in these Boloceras.

(5) Musculature.—A good character for distinguishing genera from one another is the form of the retractor muscles of the mesenteries. Of course in some families they are all of one type, but in cases where they vary the distinction is a useful one. Paraphellia is easily distinguished from Leptoteichus and Hormathia, for instance, by having among other things circumscribed retractors on its primary mesenteries, while they have diffuse ones. Isoparactis, again, is clearly marked off from Paranthus by its circumscribed retractors. There are some genera, it is true, in which, although more or less diffuse retractors are the rule, there is a tendency towards circumscription in some species. This applies to Sagartia and Aiptasiomorpha and Bartholomea, and it is interesting to note that these are, according to the evolutionary idea developed above, survivors of some of the very forms in which the transition from circumscribed to diffuse retractors would be taking place, or would have been actually accomplished in most cases, Aiptasiomorpha being one survivor of the earliest forms to lose the macro-

cneme and microcneme distinction, and Sagartia one survivor of the first set of forms to attain numerous perfect mesenteries.

The character of the individual muscle-processes of the retractors is certainly not usually of generic value, though it appears to be capable of distinguishing species from one another in some cases.

It is a clear and simple distinction to place in separate genera forms which have the longitudinal tentacular musculature ectodermal and others which have it embedded in the mesoglœa. This seems to be a natural and stable distinction. There are a few cases in which the musculature is meso-ectodermal, and these must be judged according to the requirements of the individual case.

With regard to the sphincter, it avoids confusion and is a natural arrangement to place forms with no sphincter in different genera from those with a weak mesoglœal one. If the sphincter is endodermal, its diffuseness or circumscription often form simple generic features, but if it is mesoglœal its form seems to vary too much to be of use in classification.

(6) Summary.—It has been attempted to show that the exact form of the pedal disc, the presence or absence of capitular ridges, the thickness of the body-wall, and the form of the tentacles apart from actual structural differentiation of parts of them, are characters which it would be profitable to discard as sole generic distinctions; that the distribution of gonads is not invariably a reliable generic character; and that presence or absence of definite verrucæ and acrorhagi, and of a cuticle in some cases, the presence or absence of basal swellings or other tentacular specialisations, the limitation of the cycles of tentacles to two only (at the same time as the mesenteries are normally arranged in several cycles) in some forms, the form of the retractor muscles in most cases, the position of the longitudinal tentacular musculature (whether ectodermal and mesoglœal), and the presence or absence of the mesoglœal sphincter, are more valuable characters which may usually be emphasised in distinguishing genera. I have

shown before (**79**, p. 4) that the length of the body in proportion to its height may be a valueless character in certain cases, although some aspects of the form of the body are permissible characters for differentiation. This does not, of course, survey the field of generic characters at all completely, but it provides a basis for the re-defining of the genera especially dealt with in this paper. Individual cases have, of course, their own needs, which may except them from some fairly general principle. It should of course be understood that in suggesting that certain generic characters should be allowed to lapse, I am not intending omission of any mention of them, but merely that any one of them should not be made ground for separating two given species, otherwise similar, into different genera.

X. Definitions of the New and Revised Families and of the Genera Contained in them.

Family 1. DIADUMENIDÆ, n. fam.

METRIDIINÆ as used by Annandale, 1915, pro parte.

Actiniina. Pedal disc may be well developed or so reduced as practically to form a physa. Body-wall delicate, without cuticle, pierced by cinclides. Tentacles simple, their longitudinal musculature ectodermal. Oral disc simple or branched. Mesenteries divided into macrocnemes and microcnemes, but in one genus the division not sharply marked and may be practically lost in some individuals. Six pairs of macrocnemes which bear well-developed and usually circumscribed retractors and (with some exceptions in some individuals) gonads and filaments. A variable number of microcnemes which are more or less rudimentary according to genus; they are typically rudimentary, but in one species some of them may, exceptionally, bear gonad and filament, and in another some of them may attain considerable development; but even at the best some of them are rudimentary. Acontia present. Sphincter absent or weak mesoglœal, but relationship of forms without it to forms with it quite clear.

Genera: Diadumene, Pelocœtes, Phytocœtes, Mena.

Diadumene, n. nom. (see also p. 457).

Metridium as used by Annandale (1907, p. 47, and 1915, p. 76) for M. schillerianum.

NOT Metridium, Oken.

Diadumenidæ with broad. well-developed base. Column usually with vertically-arranged rows of suckers. Cinclides scattered. A convex ring-like collar present round upper part of column in expansion. Tentacles in several cycles. Oral disc simple, retractile. Microcnemes in 3–5 cycles; in some *individuals* gonads may be found on some of them; and although some are always rudimentary, some of them may bear filament and longitudinal muscle. No mesoglœal sphincter. Retractors of macrocnemes may tend to be diffuse.

Species: D. schilleriana, Stoliczka (Sagartia schilleriana, Stolickza, 1869, p. 28. See Annandale, 1907, p. 47, etc., and 1915, pp. 72–78). (See Pl. 22, fig. 12.)

The sole recorded species of the genus Diadumene was originally described by Stoliczka as Sagartia schilleriana. It has more recently been made the subject of research by Annandale (1907, 1915), who has worked out in great detail both its anatomy and its relationships with Pelocœtes, Phytocœtes and Mena. He has included it, however, in the genus Metridium, Oken, which, though a minor matter, can hardly be correct. If we compare Metridium, as typified by M. dianthus, with S. schilleriana we find the following differences.

M. Dianthus.	S. schilleriana.
Column smooth.	Column usually with suckers.
Oral disc much lobed in adult.	Oral disc simple in adult.
Sphincter mesoglœal, well developed.	No mesoglœal sphincter.
Primary mesenteries sterile.	Primary mesenteries fertile.
Radial musculature of oral disc sometimes partly mesoglœal.	Radial musculature of oral disc ectodermal.
Mesenteries forming a graded series, not divided into macrocnemes and microcnemes, their retractors diffuse. Number of perfect mesenteries variable, although the six sterile primary pairs are often alone perfect. Imperfect mesenteries, many of them fertile.	Mesenteries here, although not fully divided into macrocnemes and microcnemes, tend that way, especially in some individuals where only the primaries are fertile. Only the macrocnemes perfect. Retractors more tending to circumscription than in M. dianthus.

These differences are not all of equal weight, but some of them are important, and taken together they show that the two forms are very different and cannot be included in the same genus. My case for allocating them to different families will be found in the earlier parts of the paper and really depends more upon the relatives of schilleriana than upon that species itself, since it is aberrant from the general tendencies of its group. A new name is therefore required for the genus containing schilleriana, which is no more a Sagartia than a Metridium, and I have proposed Diadumene for it. This name is also a convenient one to work into the name of the new family to which Diadumene and its relatives belong.

PELOCŒTES. Annandale, 1915, p. 85. (See Text-fig. 28 and Pl. 22, fig. 13.)

Diadumenidæ with the basal disc much reduced, the aboral extremity capable of assuming a physa-like appearance and shape. Column vermiform, with no collar, with suckers in the upper part arranged in vertical rows, and with cinclides also arranged in vertical rows in the upper part. The majority of the tentacles arranged in groups, each of which is placed on a flattened pedicel or outgrowth of the reduced, non-retractile oral disc. The only recorded species has thirty-six microcnemes, of which some are occasionally fertile, though as a rule they are rudimentary (Text-fig. 28). No mesoglœal sphincter.

Species: P. exul, Annandale, 1907, p. 48, etc., and 1915, pp. 72–76, 86. (See Text-fig. 28 and Pl. 22, fig. 13.)

PHYTOCŒTES, Annandale, 1915, p. 78.

Diadumenidæ with basal disc small and unmuscular, never strongly adhesive, the aboral extremity capable of assuming a physa-like shape and appearance. Column capable of considerable elongation but protean in form, smooth and very thin, the cinclides scattered in the upper part. Tentacles thread-like in full expansion, retractile. Oral disc simple. In the only recorded species there are usually twelve microcnemes, all of them almost vestigial. No mesoglœal sphincter.

Species: P. gangeticus, Annandale, 1915, pp. 72–76, 79. (See also 1907.)

MENA, n. gen.

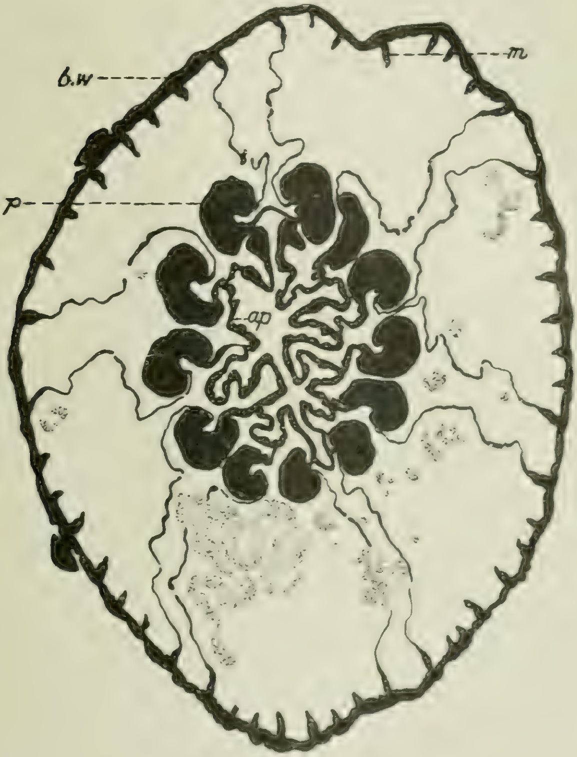
PHYTOCŒTES, Annandale, 1915, p. 78, pro parte.

Differs from Phytocœtes by having a weak mesoglœal sphincter, and, in the only recorded species, minute papillæ on the body-wall.

Species: M. chilkaea, Annandale, 1915, p. 82. (See also pp. 72–76.)

I have proposed a new genus (Mena) for the reception of Annandale's Phytocœtes chilkaeus, because it avoids confusion in identifying to have forms with no sphincter separated from those with a mesoglœal one; and the difference is one of enough importance to warrant separation. Halcampa is distinguished from Halcampoides, and Aiptasia from Aiptasiomorpha, in the same way.

TEXT-FIG. 28.



Transverse section of body of Pelocœtes exul. *ap.* Actinopharynx. *bw.* Body-wall. *m.* Microcneme. *r.* Retractor

Family 2. PHELLIIDÆ.

PHELLINÆ, Verrill, 1868, p. 324, 1869, p. 489.
PHELLIINÆ as used by Haddon, 1898, p. 452.
NOT PHELLINÆ as used by Carlgren, 1893, p. 109, except for PHELLIA itself.
PHELLIIDÆ, Bourne, 1918, p. 84.

Actiniina. Body more or less definitely divided into a middle part or scapus provided with cuticle which is sometimes deciduous; scapus may be incrusted; a usually more delicate or extensile capitulum without cuticle, and which can be introverted; and a base which may be adherent or may resemble a physa. Cinclides absent. Tentacles and oral disc simple. Longitudinal musculature of tentacles usually ectodermal, sometimes meso-ectodermal. Radial peristomial musculature may be absent. Mesenteries divided into macrocnemes and microcnemes. Six pairs (or rarely fewer and rarely a very few more than six pairs) of macrocnemes which bear circumscribed retractors, filaments, and most or all of them gonads. A variable number of microcnemes which are more or less rudimentary; they bear no gonads, but some of them may bear acontia and filaments (usually very small ones) and vestiges of longitudinal musculature; their only well-developed muscles are the parietals. Acontia present, sometimes rudimentary. Sphincter usually mesoglœal, rarely absent, sometimes divided into a thicker scapar and thinner capitular portion.

Genera: PHELLIA, DECAPHELLIA, HALCAMPACTIS, and perhaps ISOPHELLIA.

Doubtful genera which may possibly find a place here are Octophellia, Andres, and Ilyactis, Andres.

PHELLIA, Gosse, Ann. N.H., 3, ii, 193, and 1860, p. 134.

Phelliidæ with base of variable development; it may be distinctly adherent, or may be more or less inflatable and comparable to a physa, and may be somewhat reduced. Peristomial musculature absent or reduced, causing the mouth to gape (always?). Six pairs of macrocnemes, usually all fertile. Microcnemes variable in number. Acontia variable, sometimes rudimentary. Sphincter mesoglœal, typically divided into a broader portion in the scapus and a narrower one in the capitulum. Longitudinal musculature of the tentacles ectodermal, may be very powerful, or sometimes meso-ectodermal. Form of body variable; may be elongate.

Species: P. murocincta, Gosse, Ann. N.H., 3, ii, 193, and 1860, p. 135, is the type of the genus. Other species are numerous.

DECAPHELLIA, Bourne, 1918, p. 60.

Phelliidæ "with the characters of Phellia, but the capitulum has no musculature except for a mesoglœal sphincter at its distal extremity, and there are only ten complete macromesenteries[1] bearing longitudinal retractor muscles."

Species: D. psammomitra, Bourne, 1918, p. 60.

HALCAMPACTIS, Farquhar, 1898, p. 530.

Phelliidæ with a rounded aboral extremity or physa. Six pairs of macrocnemes, and in the only anatomically described species six pairs of microcnemes. Body-wall provided with minute suckers; cuticle deciduous. No sharply-defined sphincter.

Species: H. mirabilis, Farquhar, 1898, p. 530. H. dubia, Stuckey, 1909, p. 387.

ISOPHELLIA, Carlgren, 1900, p. 72. (See p. 487.)

? Phelliidæ. Basal disc not sharply marked off. Scapus long, with numerous small papillæ to which sand-grains are attached. Capitulum smaller, without papillæ, smooth. Tentacles simple, in several cycles in the adult, their longitudinal musculature ectodermal. The most proximal part of the mesoglœal sphincter sometimes separated from the remaining part. Macrocnemes of the first cycle as usual, six pairs, with gonads and strong retractors. Half the mesenteries (three couples) of the second cycle may also be macrocnemes like the primary mesenteries; the rest of the second cycle then imperfect and with weak or no distinct retractors. Third cycle consists of microcnemes. Probably more microcnemes present in distal part of body. Gonads found on those of the second-cycle mesenteries which are macrocnemes, and on at least one of the weaker second-cycle mesenteries.

Species: I. sabulosa, Carlgren, 1900, p. 72. (See p. 487.)

For a discussion of the systematic position of this genus see p. 487.

Family 3. MARSUPIFERIDÆ, n. fam.

PARACTIDÆ as used by Carlgren, 1901 and 1902, pro parte.

Actiniina. Pedal disc of variable extent. Column divided into a cuticled or encrusted scapus and a naked capitulum. No cinclides. Tentacles simple, their longitudinal musculature ectodermal. Oral disc simple. Mesenteries divided into macrocnemes and microcnemes. Six pairs of macrocnemes with powerful retractors, gonads and filaments. The microcnemes may be broad, but are devoid of gonads, filaments and definite retractors. No acontia. Sphincter mesoglœal, double or single. Brood-pouches may develop in connection with the microcnemes of the female.

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[1] Macrocnemes.

Genera: MARSUPIFER, PHELLIOMORPHA.

It is not impossible that the doubtful genera Phelliopsis, Verrill, and Cactosoma, Dan., may find a place here.

MARSUPIFER, Carlgren, 1901.

Marsupiferidæ with definite base. Column smooth but for the cuticle of the scapus, capitulum naked and introvertible. Six pairs of macrocnemes, few microcnemes. Sphincter well developed, double. Female, in the only recorded species, with six brood-pouches developed in connection with the microcnemes.

Species: M. valdiviæ, Carlgren, 1901.

PHELLIOMORPHA, Carlgren, 1902.

PHELLIA as used by Danielssen, 1887, pro parte.
ISOPHELLIA, Carlgren, 1900, p. 52, pro parte.

Marsupiferidæ with base which may not be broad. Scapus with papillæ attaching sand-grains. Six pairs of macrocnemes and a few microcnemes. Sphincter very weak, single, lying just at the bases of the tentacles.

Species: P. crassa, Danielssen. (See Carlgren, 1902.)

Family 4. METRIDIIDÆ.

METRIDINÆ, Carlgren, 1893, p. 101.

Actiniina. Definite base, which may be broad or may be small and weakly adherent. Body-wall delicate or fairly firm, but with no cuticle; pierced by cinclides. Tentacles simple or with curious spherical or ring-like or spiral protuberances either near the tip or spread over the whole tentacle; but without aboreal basal thickenings of the mesoglœa. Longitudinal musculature of tentacles ectodermal or meso-ectodermal. Oral disc simple, undulate, or lobed. Mesenteries

NOT divided into macrocnemes and microcnemes. Number of perfect mesenteries typically six pairs only, but sometimes a few more perfect mesenteries are present in certain individuals of a species; or the typical number for a species may be eight pairs. Retractor muscles diffuse or with a tendency to circumscription. Perfect mesenteries fertile or sterile, usually the latter. Acontia present. Sphincter mesoglœal, strong or weak, sometimes absent; perhaps even weak endodermal in one case, those forms in which it is absent being clearly related to those which have it.

Genera: METRIDIUM, CALLIACTIS, ADAMSIA, AIPTASIA, AIPTASIOMORPHA, HETERACTIS, BARTHOLOMEA.

Another genus which may possibly come here is Stelidiactis, Dan.

METRIDIUM, Oken, 1816.

ACTINOLOBA, Blainville, 1830.

Metridiidæ with well-developed base. Body-wall smooth, with a very distinct circular collar round the upper part, in expansion; it is less evident in the young than in the adult. Cinclides scattered in the delicate wall below the region of the sphincter. Oral disc circular in the young, but deeply and conspicuously lobed in the adult, bearing many simple tentacles; its radial musculature may be ectodermal or partly mesoglœal, though the longitudinal musculature of the tentacles is ectodermal. Perfect mesenteries very variable in number and often irregular in development. In the majority of individuals the number of perfect pairs is between six and eleven, and of these individuals a considerable number have the standard six pairs alone perfect. More rarely there are fewer than six pairs perfect, and still less often are more than eleven pairs perfect. This irregularity is probably connected with the fact that asexual reproduction, especially by means of basal fragmentation, is of frequent occurrence. The six primary pairs of mesenteries are sterile. Sphincter well developed, mesoglœal, simple. Retractors diffuse, sometimes short and broad in section.

Species: The genotype is M. dianthus, Ellis, 1768, p. 428. (See also Carlgren, 1893, p. 102, Torrey, 1898 and 1902, McMurrich, 1901, etc.)

The above definition of Metridium may perhaps prove to be too narrow and exclusive, but for the present I have drawn it up chiefly with regard to the type-species M. dianthus, of which M. marginatum and M. fimbriatum are probably synonyms (see McMurrich, 1901). Whether it will need modification when more is known of other species remains to be seen, but no forms in which the adult has a non-lobed disc or no collar should be included in it. Anyone who has seen M. dianthus alive and healthy cannot have failed to note these two striking characters. Even in the young one begins to see the collar and the plumy character of the tentacles. If it should be found that there are other species like Metridium but in which the disc in the adult remains entire, a new genus will be required for them. It is possible that M. parvulum, McMurrich, 1904, and M. canum, Stuckey, 1913, are such forms, or, on the other hand, they may be young forms. For discussion of M. schillerianum, Stoliczka, which is not really a Metridium, see p. 521.

CALLIACTIS, Verrill, 1869, p. 481.

SAGARTIA as used by Gosse, 1860, pro parte. (S. PARASITICA.)
ADAMSIA as used by M. Edw., 1857, pro parte, by Andres, 1883, p. 366, pro parte.

Metridiidæ with well-developed base. Column more or less cylindrical, not distorted; its wall may be firm or rather thick, and in some cases may form a kind of membranous investment, which is often shed, over part of the column. Cinclides in one or more horizontal rows near base of column; they may or may not be mounted on small tubercles. No collar. Margin tentaculate. Sometimes the oral and pedal discs are expanded beyond the column; the former is not lobed but may be somewhat undulate; its radial musculature may be partly mesoglœal. Tentacles simple, in more than two cycles[1] in the adult, their longitudinal musculature ectodermal or meso-ectodermal. Six pairs of perfect mesenteries which are sterile. Retractors diffuse. The species usually live on a shell inhabited by a hermit crab, but not invariably. Sphincter mesoglœal.

Species: Genotype, C. decorata, Drayton in Dana, 1849.

Others are C. variegata, Verrill, 1869, p. 481.

C. tricolor, Lesueur, 1817, p. 171 (= C. bicolor, Les., 1817, p. 171 = C. egletes, D. & M., 1866, p. 134. See also McMurrich, 1898.)

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[1] It should be understood that when "tentacles in more than two cycles" appears in a definition, it does not exclude from the genus any species which may never attain more than two cycles because of extreme smallness when adult, and does not refer to young specimens.

C. fusca, Q. & G. (fusco-rubra, Q. & G., 1833, p. 145, fusca, M. Edw., 1857, p. 281).

C. polypus, Forskål, 1775, p. 102. (See Hertwig, 1882, p. 74, and Carlgren, 1900.)

C. miriam, H. & S., 1893, p. 130, Haddon, 1898, p. 457.

C. reticulata, Stephenson, 1918, A, p. 53.

C. parasitica, Couch, 1838, p. 80. (See Gosse, 1860, p. 112; Hertwig, 1879; Faurot, several papers.) (? = C. rondeletii, D. Ch., 1828, p. 72.)

C. kroyeri, Dan., 1887, is a synonym of Allantactis parasitica.

The British representative of this genus is C. parasitica, Couch (= Sagartia parasitica, Couch). There can be no doubt that this form really is a Calliactis and not a Sagartia. It differs from Sagartia as represented by the type-species S. miniata, venusta, etc., by having—

(i) the cinclides confined to a region a little above the base;

(ii) only six pairs of perfect mesenteries instead of a larger number;

(iii) the primary mesenteries sterile instead of fertile.

Apart from this there is a great difference in details and in habit between C. parasitica and the typical Sagartias, as I can testify from personal experience; and the habitat again (on a shell inhabited by a hermit crab), although not exactly a generic character, is a feature often found in Calliactis.

ADAMSIA, Forbes, 1840, p. 181.

Metridiidiidæ with the body much distorted in the adult so as to form a wrap round a shell inhabited by a hermit crab. The basal disc is greatly extended and secretes a cuticle, formed of solidified mucus, which may extend beyond the mouth of the shell, when the latter becomes too small for the anemone, so as to form not only a support for the anemone but also a house for the crab. Cinclides towards the basal margin of the smooth body; they may be mounted on slight elevations. Oral disc not lobed. Tentacles in more than two cycles in the adult, simple. Sphincter mesoglœal. Development of mesenteries may be irregular and asymmetrical.

Species: The genotype, to which the above definition refers, is A. palliata, Bohadsch, 1761, p. 136. (See Gosse, 1860, p. 125, and several papers by Faurot.)

AIPTASIA,[1] Gosse, Ann. N.H., 3, i, 416, and 1860, p. 151.

AIPTASIOIDES, Stephenson, 1918, A, p. 51.

Metridiidæ with definite base which may vary; it may be well developed; its margin may be irregular, almost "lacerate"; or it may be quite small and weakly adherent, and able to be inflated rather like a physa. Body-form normal but variable, and may attain a long trumpet-shape. Wall delicate, with the cinclides usually in a horizontal zone near the middle of the column. Margin tentaculate. Tentacles simple and smooth, usually non-retractile; may be long, in more than two cycles in the adult, their longitudinal musculature ectodermal. Oral disc simple. Six pairs of perfect mesenteries with diffuse retractors; they may be fertile. Sphincter very weak, mesoglœal. Ectodermal longitudinal musculature may be present in body-wall and actinopharynx, at least in the uppermost part.

Species: Genotype, A. couchi, Cocks, 1851, p. 11. (See Gosse, 1860, p. 152, and this paper, p. 437.)

Other species are A. pallida, Agassiz, 1849 (MS.). (See McMurrich, 1889, Journ. Morph. and Proc. Ac. Nat. Sci. Philad.)

A. sp., McMurrich, 1889, Proc. Acad. Nat. Sci. Philad.

A. prima, Stephenson, 1918 (= Aiptasioides prima, Steph., 1918, A, p. 51).

The genus Aiptasia as here defined is more limited than it has sometimes been—limited, in fact, to the nearest relatives of the genotype A. couchi, the anatomy of which is now definitely described on p. 437. I have excluded from it forms with no mesoglœal sphincter, and also forms with specialised tentacles, because although these are closely related to Aiptasia, the possession by them of the two characters mentioned seems to necessitate generic separation in the case of the lack of a mesoglœal sphincter at any rate. The other character is mentioned again on p. 516, and under Heteractis.

AIPTASIOMORPHA, n. gen.

AIPTASIA as used for forms without mesoglœal sphincters and with smooth tentacles.

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[1] It may here be noted that Bourne has suggested classing Aiptasia with Anemonia. But since it is now known that several Aiptasias and relatives have mesoglœal sphincters, I take it that this, together with their acontia and cinclides, show their relationships to be with Metridiidæ.

Metridiidæ with definite base and normal but variable form, which may be like that of a long trumpet or pillar, or may be short. Wall more or less delicate, with cinclides which are typically arranged in a horizontal zone near middle of column, or in horizontal rows in lower parts. Margin tentaculate or with a parapet. Tentacles simple and smooth, in more than two cycles in the adult, probably usually non-retractile, their longitudinal musculature ectodermal. Oral disc simple. Typically six pairs of perfect mesenteries; they may be fertile, and their retractors may be more or less circumscribed. No mesoglœal sphincter.

Species: A. paxi, n. nom. (A. couchii, Pax, 1909, p. 337.) (See this paper, p. 439.)

A. minima, Stephenson, 1918, A, p. 49.

A. leiodactyla, Pax, 1910, p. 198.

A. diaphana, Rapp, 1829.

In 1918 (**79**, p. 51) I instituted a genus Aiptasioides to receive forms resembling Aiptasia but with a mesoglœal sphincter, following Pax in limiting Aiptasia to forms with no sphincter. At that time A. couchii, the genotype of Aiptasia, had not been anatomically described from British specimens; but now that it has proved (see p. 438) to have a weak mesoglœal sphincter, the name Aiptasia must apply to the forms which I included in Aiptasioides as well as to A. couchii, and a new name is required for sphincterless forms. I propose Aiptasiomorpha, and confine it at present to forms with smooth tentacles.

HETERACTIS, Milne-Edwards, 1857.

AIPTASIA as used for A. lucida by some authors.

Metridiidæ with definite base. Cinclides in a horizontal zone near middle of column. Margin tentaculate. Tentacles in probably more than two cycles in the adult, not fully retractile; they possess on their surface a number of scattered spherical or reniform protuberances, which are hollow outpushings of the wall of the tentacle, differing histologically from the rest of the tentacle chiefly in that they possess many nematocysts and have reduced longitudinal musculature. Longitudinal musculature of tentacles ectodermal. Oral disc not lobed. Six pairs of perfect mesenteries. Diffuse retractors. Sphincter weak mesoglœal.

Species: H. lucida, D. & M., 1860.

It has seemed best (see p. 516) to separate forms with modified from those with normal tentacles, and this involves the restoration of the genus Heteractis. I have defined it to fit H. lucida, as described by McMurrich, 1896, p. 182. There remains the difficulty that H. aurora is really the type of Heteractis, and if this proves to be something different from H. lucida the latter will need another name. Heteractis differs from Bartholomea in that it has a mesoglœal sphincter. Pax describes (1910, p. 201) an anemone which he identifies as lucida, but it has no sphincter, and from his figure of a tentacle it would seem that the thickenings are rather different from those of McMurrich's species. Perhaps Pax's anemone is something other than lucida, and should be included under Bartholomea as defined below.

BARTHOLOMEA, Duch. & Mich., 1866.

AIPTASIA as used for A. ANNULATA, etc.

Metridiidæ with definite base. Body-wall delicate, with cinclides typically in a horizontal zone near middle of column, which may be fairly long. Margin tentaculate. Oral disc not lobed. Tentacles in more than two cycles in the adult, their longitudinal musculature ectodermal; they may be long and are not fully retractile. The tentacles have spiral or incompletely annular raised bands on their surface, which are of the nature of batteries of nematocysts; these bands may occur throughout the extent of the tentacle, or near the tip only. Perfect mesenteries, six, seven, or eight pairs. Retractors diffuse, sometimes rather restricted. Primary mesenteries usually sterile. No mesoglœal sphincter.

Species: Genotype, B. annulata, Lesueur, 1817, p. 172. (See also McMurrich, 1889, Journ. Morph., and Pax, 1910, p. 206.)

B. peruviana, Pax, 1912, p. D. 18.

? B. tagetes, D. & M., 1866, p. 133. (See McMurrich, 1889, Journ. Morph., and Pax, 1910, p. 203.)

In separating from Aiptasia forms with specialised tentacles (see p. 516), it has been necessary to revive two old genera—Heteractis for forms with a mesoglœal sphincter and Bartholomea for those with no sphincter. The genus Bartholomea was established in 1866 and included B. solifera (=annulata) and B. tagetes. As these are the two species with which we are mainly concerned at the moment Bartholomea is evidently the right name for them. I have put a query before the name of B. tagetes because I am not sure whether it belongs here or in Aiptasiomorpha. McMurrich describes the tentacles as smooth, but Pax states that the thickenings are present but inconspicuous and flat.

Family 5. CHONDRACTINIIDÆ.[1]

CHONDRACTININÆ, Haddon, 1889, p. 304.
CHONDRACTINIINÆ, Haddon, 1898, p. 458.
PHELLINÆ as used by Carlgren, 1893, p. 109, except PHELLIA.
NOT PHELLINÆ, Verrill, 1868.

Actiniina. Definite base, which may be flat and adherent, or may clasp a cylindrical object or form a mud-enclosing cup; more than one of these types may occur in one and the same species. Body-wall variable, frequently tough and cartilaginous and sometimes very thick; may be uninterrupted or may be divided into two regions—a main lower scapus and a submarginal capitulum. These regions may both be smooth; the lower may be encrusted with sand, or the scapus may develop a smaller or larger number of prominent or insignificant tubercles and may also possess cuticle; the capitulum may bear ridges or crests which may break up into tubercles, but it is almost always free from cuticle. No cinclides.[2] Tentacles simple or with aboral basal swellings of mesoglœa, their longitudinal musculature ectodermal or meso-ectodermal. Oral disc simple or bilobed.

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[1] There is not now any genus called Chondractinia, since that name has been shown to be a synonym of Hormathia. But that does not provide any reason why the name Chondractiniinæ of Haddon should not be used as the foundation of the name of the new family—it is not misleading and is very appropriate to many of the forms included in the family.

[2] With regard to this it may be noted that although the members of this family have not, so far as we know, any true cinclides, there is at least one record of perforations of the wall which, although probably not really cinclides, were rather curious. An account of these will be found in **80**, pp. 144–145.

Mesenteries NOT divided into macrocnemes and microcnemes. Only six (or rarely a very few more) pairs of mesenteries perfect. Primary mesenteries sterile. Retractors as a rule diffuse, but may be restricted or circumscribed. Acontia present, but may be so reduced as to be rudimentary. Sphincter mesoglœal, usually well developed.

Genera: HORMATHIA, ACTINAUGE, PARAPHELLIA, SAGARTIOMORPHE, LEPTOTEICHUS, CHONDRODACTIS, and probably PHELLIACTIS.

Other genera of doubtful standing which may possibly find a place here are Ammonactis, Verrill, Kodioides, Dan., Allantactis, Dan., Anthosactis, Dan.

HORMATHIA, Gosse, Ann. N.H., 3, iii, 47, 1860, p. 218.

TEALIA, Gosse, Ann. N.H., 3, i, 417, 1860, p. 205, pro parte (T. digitata).

BUNODES, Gosse, Ann. N.H., 3, ii, 194, 1860, p. 189, pro parte (B. coronata).

CEREUS as used by Hertwig, 1882, p. 76 (C. SPINOSUS).

NOT CEREUS, Oken.

PHELLIA as used by Hertwig, 1882, p. 80 (P. PECTINATA), and 1888, p. 24 (P. SPINIFERA).

NOT PHELLIA, Gosse.

CHONDRACTINIA, Lütken, 1860, p. 190.

CHITONACTIS, Fischer, 1874, p. 226.

CHITONANTHUS, McMurrich, 1893, p. 189.

Chondractiniidæ with variable base, which may be an adherent disc or may form a more or less fully developed cup enclosing mud, or may be long and narrow, clasping a spine-like support, in which case its edges may fuse where they meet; more than one of these types may occur in one and the same species. Body-wall variable, often tough and cartilaginous, sometimes thick. Column divided more or less definitely into scapus and capitulum. Except in small and in rare smooth specimens the scapus possesses at least some development of solid tubercles. These may form one single circle round its distal margin, or they may be more widely distributed; in the latter case they may be without definite arrangement, or there may be a tendency to arrangement in vertical rows, which may be carried out definitely as regards at least some of them, in which case the rows are as a rule in multiples of six, rarely of seven or eight. Typically about twelve (or a multiple) "coronal" tubercles are recognisable where scapus and capitulum join, though they may not be different from the other tubercles and may merge into the ridges of the capitulum; the scapus may or may not be provided with cuticle. Capitulum nearly always free from cuticle; may be smooth, or may be provided with longitudinal ridges which may be distinct or feebly developed and vary in form, sometimes being broken up into tubercles themselves; they sometimes appear on incipient contraction, being absent in expansion; when present they are most often about twelve (or a multiple) in number. Tentacles simple, in more than two cycles in the adult, their longitudinal musculature ectodermal. Oral disc not lobed; its radial musculature may be partly mesoglœal. Typically six pairs of perfect mesenteries, rarely seven or eight pairs. Retractors diffuse, but may be very well developed.

Species: Genotype, H. margaritæ, Gosse, Ann. N.H., 3, iii, 47, and 1860, p. 219. (See also Haddon, 1889, p. 310.)

Others are H. andersoni, Haddon, 1888, p. 251.

H. digitata, Müller, 1776, p. 231. (See Haddon, 1889, p. 306, and Carlgren, 1893, pp. 110 and 138.) (= Tealia digitata, Gosse.)

H. coronata, Gosse, Ann. N. H., 3, ii, 194, 1860, p. 202.

H. marioni, Haddon, 1889, p. 313.

H. nodosa, Fabr., 1780, p. 350. (See Haddon, 1889, p. 308, and Carlgren, 1893, pp. 115, 138.)

H. pectinata, Hertwig, 1882, p. 81. (See also McMurrich, 1893, p. 190.) (= H. spinifera, Hertwig, 1888, p. 24.)

H. exlex, McMurrich, 1904.

H. castanea, McMurrich, 1904.

H. longicornis, Verrill, 1882, p. 222.

H. spinosa, Hertwig, 1882, p. 76.

The long definition of the above genus will show how variable a one it is in some respects. Moreover, many of the variations mentioned may be exhibited by different specimens of one and the same species, so that generic characters cannot be made of them. McMurrich (1893, p. 209) and Haddon (1898, p. 459) have already pointed out that Chondractinia and Chitonactis cannot be distinguished from Hormathia on the basis of the nature and arrangement of the tubercles. It is also clear that McMurrich's genus Chitonanthus cannot stand, since the presence or absence of capitular ridges is not constant for some species (see p. 512). The species assigned to Phellia and Cereus by Hertwig, moreover, really belong to this genus; Hertwig did not correctly interpret the two genera in question, as has been pointed out before by Haddon and McMurrich. Bunodes minuta, Hertwig, has also been placed in Hormathia by Haddon

(1898, p. 459; 1889, p. 312). It is probably a member of this genus, but has only *two* cycles of tentacles and only *two* pairs of perfect sterile mesenteries instead of six. The second of these features may be an individual abnormality or both may be due to the small size of the animal, but I prefer to leave the species out of the genus till more is known of it.

ACTINAUGE, Verrill, 1883, p. 50.

Chondractiniidæ with adherent or mud-clasping base, both sorts found in the same species. Body-wall typically cartilaginous, very thick or very thin according to state of distension, etc. Column divided more or less definitely into scapus and capitulum. Scapus tuberculate, the tubercles with or without cuticle, irregularly arranged or with a tendency to arrangement in vertical rows; coronal tubercles or coronal rows of tubercles about twelve or a multiple of twelve, more or less distinct, sometimes merging into capitular ridges. Capitulum nearly always without cuticle, usually ridged, but not invariably, the ridges very variable in development, typically about twelve or a multiple. Tentacles in more than two cycles in the adult, their longitudinal musculature ectodermal. Each tentacle (or sometimes each tentacle of the inner cycles) has a solid mesoglœal swelling on its aboral side at the base; the swellings may be very large, or they may be so reduced as to be almost suppressed, both states occurring in different individuals of one species; but they are always present; there is a definite connection between the ridges of the capitulum and the bases of some of the tentacles—in A. richardi, for instance, the ridges appear to be often due to extensions of the basal swellings of the fourth-cycle tentacles which run over the edge of the disc and down the capitulum. Oral disc not lobed. Six pairs of perfect mesenteries. Retractors diffuse, may be well developed.

Species: Genotype, A. verrillii, McMurrich, 1893, p. 184. (= Urticina nodosa, Verrill, 1873, p. 440.)

A. fastigata, McMurrich, 1893, p. 187. (= Actinauge nodosa var. coronata, Verrill, 1883.)

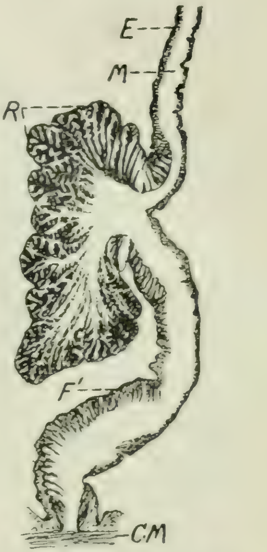
A. richardi, Marion, 1882, p. 460. (See Haddon, 1889, p. 319, and Stephenson, 1918, B, pp. 148–154.)

PARAPHELLIA, Haddon, 1889, p. 321. (See Text-figs. 29 and 30.)

Chondractiniidæ with well-developed base which may be widely expanded. Column divided into scapus and capitulum; scapus smooth or slightly corrugated, without cuticle (always?), but sometimes encrusted with sand or with a thin coating of hardened mucus in which sand may be embedded or a thick wrinkled bark-like coating; capitulum narrow and smooth. Tentacles simple, in more than two cycles in the adult, their longitudinal musculature ectodermal. Oral disc simple, its radial musculature ectodermal. The six pairs of perfect mesenteries have strong circumscribed retractors, whereas those of the second cycle are diffuse. (See Text-figs. 29 and 30.)

Species: P. expansa, Haddon, 1886, p. 616, 1889, p. 321. (See Text-figs. 29 and 30.)

TEXT-FIG. 29.



Primary mesentery of Paraphellia expansa. *CM.* Endodermal circular musculature. *E.* Endoderm. *F'.* Mesogloeal processes for muscle. *M.* Mesoglœa. *R.* Retractor.

The above definition is drawn up so that of the three species which have been referred to the genus Paraphellia it includes only the one for which the genus was originally founded (P. expansa). I have intentionally excluded the others (P. lineata and P. hunti) because they seem to me to be sufficiently different from P. expansa to claim generic distinction. P. expansa, with which I am personally acquainted, is a very distinct form, and its powerful circumscribed retractors are enough in themselves to separate it from forms with diffuse ones. The limits of the genus Sagartiomorphe, Kwiet., are not at present very clear, but it would seem that P. lineata and P. hunti would be better placed there than in Paraphellia, and consequently I have included them in Sargartiomorphe pending further knowledge. It is not impossible that they will finally need a distinct genus to themselves on account of the nature of the margin.

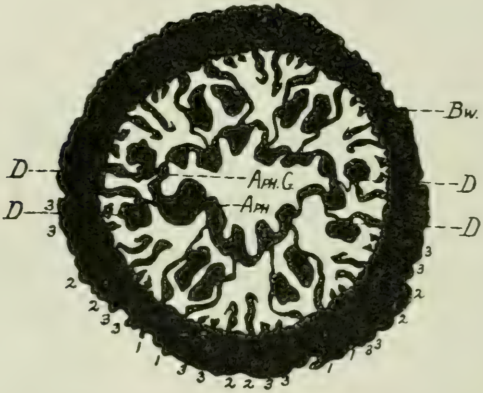
SAGARTIOMORPHE, Kwietniewski, 1898, p. 396.

PARAPHELLIA, as used by Haddon in 1893, pp. 129–130, pro parte, and in 1898, pp. 460-462, pro parte.

Chondractiniidæ with definite base. Column smooth, without cuticle its upper margin may be raised into a fold. Tentacles simple, in more than two cycles in the adult, their longitudinal musculature ectodermal. Oral disc simple, its radial musculature ectodermal. Perfect mesenteries with diffuse retractors.

Species: Genotype, S. carlgreni, Kwietniewski, 1898, p. 396. To this I add S. hunti, H. & S., 1893, p. 129, 1898, p. 461, and S. lineata, H. & S., 1893, p. 130, 1898, p. 462. (See note under Paraphellia.)

TEXT-FIG. 30.



Transverse section of body of Paraphellia expansa. *APH.* Actinopharynx. *APH.G.* Actinopharyngeal groove. *BW.* Body-wall. *D.* Directive mesentery. Numbers indicate mesentery-cycles.

LEPTOTEICHUS, Stephenson, 1918, A, p. 57.

Chondractiniidæ with definite base. Column not divided into scapus and capitulum, smooth, without tubercles or cuticle, with a tentaculate margin. The animal may attain large size and firm, tough, very thick wall. Tentacles in more than two cycles in the adult, devoid of basal swellings; they may be large and stout; their longitudinal musculature is almost completely ectodermal, but the radial musculature of the oral disc is partly mesoglœal. Oral disc simple. Six pairs of perfect mesenteries with quite diffuse retractors. The processes of the mesenterial musculature may be stout and finger-like. Acontia so much reduced as to be rudimentary.

Species: L. insignis, Stephenson, 1918, A, p. 57.

The features which distinguish Leptoteichus from Sagartiomorphe are not very striking when reduced to a bare definition. The most definite of them are the rudimentary nature of the acontia in Leptoteichus and the partially mesoglœal character of its radial musculature. Its tentaculate margin also marks it off at least from S. carlgreni. But a study of Leptoteichus "in the flesh" seems to show that it is different in its general make-up from the small Sagartiomorphe-forms, and in my belief it merits generic distinction. It is a large deep-water Antarctic form.

CHONDRODACTIS, Wassilieff, 1908.

Chondractiniidæ with base of variable form; it may be adherent, may clasp a spine, or may form a mud-enclosing hollow. Column typically with tough cartilaginous wall which varies, but may be very thick; it may bear rounded or pointed tubercles without very regular arrangement as a rule; there is at least frequently no cuticle; if there is any distinction into scapus and capitulum it is not very well marked; the capitulum may bear irregular ridges: margin tentaculate. Tentacles never in more than two cycles at the margin of the disc, even in the adult; they have thickenings or swellings of mesoglœa on the aboral side at the base; their longitudinal musculature is ectodermal. Oral disc simple or bilobed with the two lobes able to fold up against one another; its radial musculature meso-ectodermal or ectodermal. Six pairs of mesenteries or a very few more are perfect, and the retractors are diffuse or somewhat restricted.

Species: C. magna, Wassilieff, 1908.
C. japonica, Wassilieff, 1908.
C. duplicata, Stephenson, 1918, B, p. 142.
C. coccinea, Stephenson, 1918, B, p. 136.
C. pulchra, Stephenson, 1918, B, p. 139.

C. crassa, Wassilieff, 1908, is of doubtful standing since its acontia have not yet been discovered.

PHELLIACTIS, Simon, 1892.

Chondractiniidæ with smooth body-wall. Size may be large and wall may be very thick. Scapus with a very delicate cuticle. All tentacles with a thick abaxial swelling. Capitulum naked.

Species: P. hertwigii, Simon, 1892, p. 75.

I am unable to give a full diagnosis of this genus because I could nowhere obtain a copy of the original description of it. The details given are taken from Haddon, 1898, p. 462.

Family 6. ACTINOSCYPHIIDÆ,[1] n. fam.

PARACTIDÆ, as used by some authors, pro parte (e. g. Stuckey, 1909. Carlgren. 1901. McMurrich,1893, Stephenson, 1918, A and B).
NOT part of the original Paractidæ of Hertwig, 1882.

Actiniina. Definite base of variable form; it may be concave, enclosing mud, or much reduced, or adherent, or may clasp a spine. Body-wall variable, usually smooth, sometimes with small hollow papillæ and partially encrusted. No cinclides. Tentacles simple or with aboral basal swellings, their longitudinal musculature ectodermal. Oral disc simple or undulate or bilobed. Mesenteries NOT divided into macrocnemes and microcnemes. Only six pairs of perfect mesenteries, and these may be sterile or may bear gonads as well as some or all of the others. Retractors usually diffuse, rarely circumscribed on the primary mesenteries. No acontia. Well-developed or weak mesoglœal sphincter.

Genera: ACTINOSCYPHIA, PARANTHUS, ISOPARACTIS, ? LILLIELLA.

ACTINOSCYPHIA, n. nom. (See Text-fig. 31.)
ACTINERNUS, Verrill, 1879, pro parte.
ACTINERNUS, as used by McMurrich, 1893, for A. PLEBEIUS.

Actinoscyphiidæ with pedal disc of variable extent. It may be adherent, or may be so reduced as to be almost absent; it may be concave, secreting a cuticle and enclosing mud, or may embrace a cylindrical object. Column-wall smooth, sometimes so thick that it forms a jelly-like coating to the body (see Text-fig. 31), so that the general appearance may resemble that of a Scyphozoan. Tentacles in the adult arranged in two cycles only at the margin of the oral disc, and provided with aboral basal swellings of mesoglœa, which, at least in the case of the outer cycle, are continuous with the body-margin.

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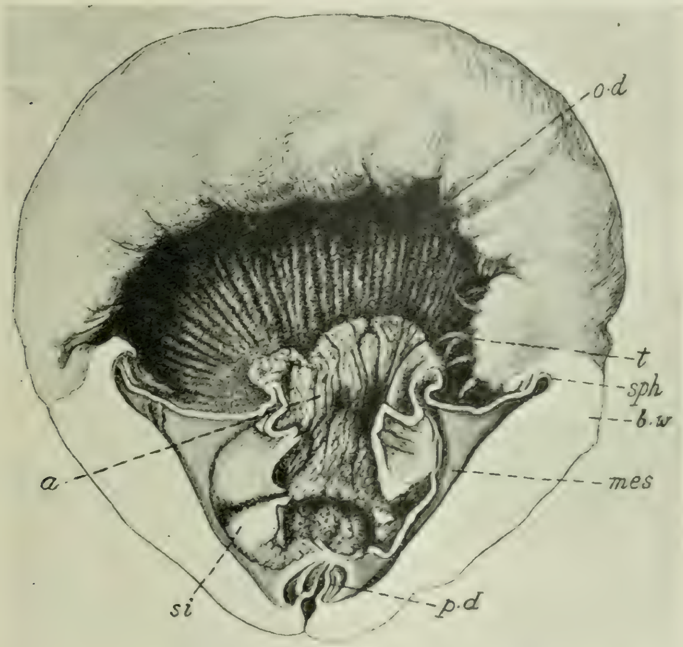
[1] If it should be thought that this (or indeed any other) family contains forms too widely different from each other as regards general external form and appearance, a study of the genera Actinoscyphia, Actinernus (Porponia), and Polysiphonia will help to show how very misleading externals are when it is a case of finding out relationships. These three genera are curiously similar superficially, but anatomically are so different that they belong to three different families—Actinoscyphiidæ, Endocœlactidæ and Paractidæ—and seem quite unrelated to one another. Possibly the deep water habitat accounts for the similarity to some extent. (See p. 488 et seq.)

Longitudinal musculature of tentacles and radial musculature of oral disc entirely ectodermal. Oral disc wide, undulate, or lobed, sometimes bilobed. The six pairs of perfect mesenteries are sterile and have weak diffuse retractors. The animals may attain large size.

Species: Genotype, A. saginata, Verrill, Amer. Journ. Sci., 3, xxiii, no. 4, p. 225. (See Stephenson, 1918, B, pp. 127–131.)

Text-fig. 31.



Actinoscyphia aurelia vertically divided, one half of the specimen, showing anatomy. *a.* Actinopharynx. *b.w.* Body-wall. *mes.* Mesentery. *o.d.* Oral disc. *p.d.* Pedal disc. *si* Actinopharyngeal groove. *sph.* Sphincter. *t.* Tentacle.

A. plebeia, McMurrich, 1893, p. 166.
A. aurelia, Stephenson, 1918, B, p. 131. (See Text-fig. 31.)

In a paper on some Irish Actiniaria (80), published in 1918, I, defined the genus Actinernus, Verrill, practically as above, pointing out at the same time that the name might have to be changed if it should prove that the type-species of Verrill's genus (A. nobilis) was a Porponia. In a paper also published in 1918, but which I did not see till my paper was printed, Carlgren has shown that A. nobilis is indeed a Porponia, so that the name Actinernus must belong to A. nobilis and other species like it, and Porponia becomes a synonym of it. But Actinernus (= Porponia) is quite unrelated to the three forms whose specific names are saginatus, plebeius and aurelia, belonging, in fact, to the Endocœlactidæ. A new name is therefore required for the genus which is to contain the three last species, and I suggest Actinoscyphia. The name refers to the cup-like form of body often found in the genus.

PARANTHUS, Andres, 1883, p. 472.

Actinoscyphiidæ with definite base. Column smooth; capitular ridges may occur; margin indistinct. Tentacles simple, in more than two cycles in the adult, their longitudinal musculature ectodermal. Oral disc simple. Six pairs of perfect mesenteries which may or may not be fertile and which bear diffuse retractors.

Species: Genotype P. chromatoderus, Schm., 1852, p. 15. (See Maguire, 1898.)

? P. lineolatus, McMurrich, 1893 (= A. lineolata ?, Couthouy in Dana, 1846). (See McMurrich, 1893, p. 162, and McMurrich, 1904.)

? P. nivea, Lesson. (See McMurrich, 1904.)

I refer the species lineolatus with some hesitation to this genus. I am not acquainted with actual specimens of it, but cannot find any very valid reason for excluding it. More study of the species, however, is desirable. But whether it belongs to Paranthus or not, it should be placed in the family Actinoscyphiidæ and removed from the genera Paractis and Pycnanthus, in both of which it has been placed at different times. Its "capitular ridges" are a character of little value as an indication of relationship (see p. 512), and its six pairs of perfect mesenteries only and its ectodermal longitudinal tentacular musculature quite remove it from Paractis and Pycnanthus, which have numerous perfect mesenteries and mesoglœal longitudinal tentacular muscles.

The other species referred with a query to the genus, P. nivea, must similarly be removed from Paractis because of its ectodermal longitudinal tentacle-muscles, but it is not quite certain whether six pairs of mesenteries or more are perfect; if six, then the species must apparently be reckoned as a Paranthus; if numerous pairs, it probably belongs to Parantheoides, unless other differences should be demonstrated which will form for it a new genus near one of those just mentioned.

ISOPARACTIS, n. nom.

PARACTIS, as used by Stuckey, 1909, p. 387, and 1913, p. 132, pro parte.

Actinoscyphiidæ with definite base. Smooth body-wall. Tentacles simple, in more than two cycles in the adult, their longitudinal musculature ectodermal. Oral disc simple. Six pairs of perfect mesenteries which bear strong circumscribed retractors and are mostly fertile. The other mesenteries are feebly developed, but are fertile.

Species: I. ferax, Stuckey, 1909, p. 387; 1913, p. 132.

The only hitherto recorded species of this genus was originally described by Stuckey (1909, p. 387; 1913, p. 132—corrections of first description in this second paper) as Paractis ferax. In re-defining the genera it has been unavoidable to remove it from the genus Paractis, and on the sum of its main features it seems to find its place in this family. Paractis is a genus the members of which have numerous perfect mesenteries, mesoglœal longitudinal tentacle-muscles and diffuse retractors. In I. ferax there are only six pairs of perfect mesenteries, and these bear strong circumscribed retractors and the longitudinal tentacular musculature is ectodermal. I have therefore suggested a new genus, Isoparactis, for the species ferax.

LILLIELLA, Stephenson, 1918, A, p. 33.

Actinoscyphiidæ (?) with definite base. Column feebly divided into scapus and capitulum, wall thick in the only recorded specimen of the only known species; scapus with poorly developed patchy incrustation, not a true cuticular sheath. Mesoglœa of scapus contains numerous lacunæ of all shapes and sizes; some of these are so large and so near the surface that they form externally visible hollow papillæ. Tentacles simple, probably in more than two cycles in the adult, their longitudinal musculature probably ectodermal. Oral disc simple. Retractors of the six pairs of perfect mesenteries probably diffuse.

Species: L. lacunifera, Stephenson, 1918, A, p. 33.

The position of this genus is as yet uncertain because the only known specimen was too badly preserved to allow of certainty as to presence or absence of acontia. If the latter should be discovered it would go to the Chondractiniidæ.

Family 7. SAGARTIIDÆ, sensu stricto.

SAGARTIADÆ, Gosse, 1858, p. 415, 1860, p. 9, pro parte.
SAGARTINÆ, Verrill, 1869, p. 477, pro parte.
SAGARTINÆ, as used by Carlgren, 1893, p. 87.
SAGARTIINÆ, as used by Haddon, 1898, p. 448.

Actiniina. Definite base. Body-wall more or less delicate, without cuticle, pierced by cinclides. Tentacles simple, their longitudinal musculature typically ectodermal. Oral disc simple or undulate. Mesenteries NOT divided into macrocnemes and microcnemes. Perfect mesenteries more than six pairs (except in very rare exceptional cases), usually numerous, the retractors more often diffuse, frequently strong diffuse. Some at least of the primary mesenteries fertile as well as others. Acontia present. Sphincter mesoglœal, often well developed.

Genera: SAGARTIA, CEREUS, ARTEMIDACTIS.

GEPHYRA, Von Koch, may also come here, and may prove to be simply a small SAGARTIA.

NEMACTIS, M. Edw., may also, possibly, find a place here.

SAGARTIA, Gosse, 1855, 1860, p. 25.

SAGARTIA, Gosse, 1855, pro parte.

SAGARTIA, Gosse, 1860, excluding "S. PARASITICA," which is a CALLIACTIS, and "S. BELLIS," which is CEREUS PEDUNCULATUS, and probably S. (?) CHRYSOSPLENIUM. Including CYLISTA, Gosse, 1860, p. 123, and THOE, Gosse, 1860, p. 122.

Sagartiidæ with definite base. This is not always used for adherence, and the animal may live free for a time or buried in mud or sand. Column soft and smooth, but for minute corrugations which may disappear on distension, and for suckers which are present in some species and may be capable of attaching foreign objects to themselves. Margin tentaculate. Cinclides scattered in certain parts of the body or generally distributed: they may be endodermal evaginations or ectodermal invaginations (see pp. 451–455); both kinds may occur in one animal; they are not always actual perforations, but may be very thin places definitely organised (see pp. 454–455). Tentacles simple, not especially numerous, in more than two cycles in the adult, their longitudinal musculature ectodermal; their arrangement may be hexamerous, pentamerous, heptamerous, octamerous or irregular. Oral disc not lobed, though it may be rather undulate in some specimens of a species at certain times, and not widely expanded, though it may be broad and somewhat exceeding the middle part of the column; its radial musculature ectodermal. Gonads appear on mesenteries of the first cycle as well as others. Number of perfect mesenteries variable; twelve pairs or more typically, but sometimes fewer; their arrangement may be hexamerous, pentamerous, heptamerous, octamerous or irregular. Retractors vary from weak diffuse to powerful diffuse or even somewhat circumscribed.

Species: Genotype, S. MINIATA, Gosse, 1853, p. 127; 1860, p. 41. (See this paper, p. 439.)

S. venusta, Gosse, and S. NIVEA, Gosse, are near relations.

S. viduata and S. undata, Müller, are described anatomically by Carlgren, 1893.

Species numerous.

Sagartia is a genus needing special study in itself. A large number of forms have been assigned to it, but the anatomy of some of them is insufficiently known. It is quite likely that when more is known of some of these they will be removed from the genus and placed in one of the genera of the Choriactidæ or Chondractiniidæ. It is quite possible, for instance, to have a form externally like Sagartia, but differing anatomically by having no cinclides or by having a different arrangement of mesenteries. It is also possible that Sagartia itself, when adventitious forms have been removed from it, may need subdivision, but at present we hardly know enough about the structure of cinclides and retractor muscles, and so on, in the genus to make any change. It is seen, however (see p. 455), that two different kinds of cinclides may occur in one and the same specimen of a species. I have not accepted the subdivision of the genus sometimes made into Sagartia, Thoe and Cylista, because intimate acquaintance with living representatives of all three has convinced me that it would be unwise to do so unless marked anatomical differences should subsequently turn up. Carlgren (1893, p. 88) has already ranked them as synonyms of Sagartia.

CEREUS, Oken, 1815, p. 349.
HELIACTIS, Thompson, 1858 (P. Z. S., xxvi, p. 145, and A. M. N. H. (3), ii, p. 229).
SCYPHIA, Gosse, 1860, p. 123.

Sagartiidæ with definite base. Column soft and smooth but for minor corrugations, with suckers capable of attaching foreign bodies to themselves. Cinclides scattered. Margin tentaculate. Tentacles simple, numerous, in more than two cycles in the adult. Oral disc variable in form, but capable of widely exceeding the rest of the body; the lower part of the column is sometimes elongate and stem-like (though by no means always), and the upper part expands to meet the edge of the disc; the latter may be flat and circular or vase-like or undulate, but not permanently lobed. Gonads appear on primary as well as other mesenteries, and ova and spermatozoa may occur in one and the same mesentery at the same level. Retractors well developed, diffuse.

Species: The above definition applies to the genotype, C. pedunculatus, Pennant, 'Br. Zool.,' iv, 102. See also Gosse, 1860, p. 27, and Haddon, 1889, p. 302, and 1898, p. 451. (Synonym, Sagartia bellis, Ellis, 'Zooph.' 2, Gosse, 1860, p. 27.)

ARTEMIDACTIS, Stephenson, 1918, A, p. 40.

Sagartiidæ with definite base. Wall smooth for the most part, delicate for the size of the animal (which may be large), sometimes with slight and inconspicuous nodulations near the margin. Column cylindrical below, but widely expanded above so that the oral disc greatly exceeds the column, though it is not lobed. No definite verrucæ or collar, but the margin is more or less distinct and may form a distinct parapet. Cinclides irregularly distributed. Tentacles simple, numerous, in several cycles in the adult, their longitudinal musculature ectodermal. Musculature of mesenteries weak and forming no distinct retractors; its processes, in the one recorded species, thick and lobe-like. Mesenteries of all cycles bear gonads. In the recorded species the mesenterial filament of each mesentery is confined to a certain definite area, variable in position according to cycle, above and below which the edge of the mesentery is quite free, except in the case of the numerous perfect mesenteries, where part of it may be attached to the actinopharynx.

Species: A. victrix, Stephenson, 1918, A, p. 41.

This genus is not altogether dissimilar from Sagartia and Cereus, but the aggregation of small differences makes it a very distinct form, easily recognised. In its expanded disc it resembles Cereus, but in its feeble mesenterial musculature is quite unlike it. Its body-margin is also more distinct than in either of the other two genera, and may form a distinct parapet—perhaps would always do so in life. It is a large animal living in deep water. The distribution of its mesenterial filaments, though not confined to this genus, is curious.

Family 8. CHORIACTIDÆ, n. fam.

SAGARTINÆ as used by McMurrich, 1904, pro parte.
METRIDIINÆ as used by Haddon, 1898, pro parte.

Actiniina. Definite base. Body-wall may be somewhat cartilaginous, may be smooth or tubercled but lacks cinclides; cuticle present or absent. Tentacles simple, their longitudinal musculature typically ectodermal. Oral disc simple, may be somewhat undulate. Mesenteries NOT divided into macrocnemes and microcnemes. More than six pairs of mesenteries perfect, usually twelve or more pairs perfect. Gonads probably not borne by primary mesenteries. Retractors diffuse. Acontia present. Sphincter mesoglœal.

Genera: CHORIACTIS and probably MITACTIS.

CHORIACTIS, McMurrich, 1904.

Choriactidæ with definite base. No cuticle. Column may be thick-walled, and may be more or less tuberculated, the tubercles arranged as ridges in the upper part, or there may be simply ridges, and the tubercles may be almost wanting in small specimens. Margin tentaculate. Tentacles simple, in more than two cycles in the adult, their longitudinal musculature ectodermal. Oral disc not lobed, its radial musculature ectodermal. Retractors diffuse. In the only recorded species which had developed gonads, these were absent from the mesenteries of the two oldest cycles.

Species: Genotype, C. impatiens, Couthouy in Dana, 1849, McMurrich, 1904.

C. crassa, McMurrich, 1904, is the only other known species.

MITACTIS, Haddon & Duerden, 1896, p. 162.

Choriactidæ with definite base. Column smooth, with cuticle. Tentacles simple, in more than two cycles in the adult. Retractors diffuse. Oral disc not lobed.

Species: M. australiæ, Haddon & Duerden, 1896, p. 162.

M. similis, Haddon & Duerden, 1896, p. 163.

This genus is not yet fully described but probably its position is here, and I therefore include it tentatively in the Choriactidæ.

Family 9. PARACTIDÆ, Hertwig, sensu stricto.

PARACTIDÆ, Hertwig, 1882, p. 41.

ACTINOSTOLIDÆ + PARACTIDÆ, pro parte, Carlgren, 1893, pp. 64 and 137.

PARACTIDÆ as used by various authors, pro parte (e.g. McMurrich, 1893 and 1904; Stuckey, 1909; Stephenson, 1918 A and B).

Including LIPONEMIDÆ, Hertwig, 1882, p. 63, pro parte.

Including SICYONIDÆ, Hertwig, 1882, p. 97, as applied to Sicyonis.

Actiniina. A definite but variable base which may be adherent, may be concave and clasp mud. Body-wall thick or thin, often cartilaginous, smooth or ridged or tuberculated or verrucose; margin tentaculate or well marked, sometimes provided with a collar. No cinclides. Tentacles simple or with thickening of the mesoglœa of the base or with aboral basal swellings of mesoglœa; longitudinal musculature typically entirely mesoglœal, but may be meso-ectodermal or even ectodermal, and may be reduced or absent in part of each tentacle. Oral disc simple or lobed or undulate. Mesenteries NOT divided into macrocnemes and microcnemes. Perfect mesenteries more than six pairs, usually numerous. They may or may not bear gonads, and their retractors, though rarely circumscribed, are usually diffuse. No acontia. Sphincter mesoglœal, often well developed.

Genera: PARACTIS, CYMBACTIS, HORMOSOMA, ALLOACTIS, ANTHOLOBA, TEALIDIUM, PARANTHEOIDES, SICYONIS, ACTINOSTOLA, CATADIOMENE, STOMPHIA, OPHIODISCUS, POLYSIPHONIA, and probably PSEUDOPARACTIS.

Some doubtful genera which may possibly be included here in the future are Raphactis, Verrill; Archactis, Verrill; Ammophilactis, Verrill; Paractinia, Andres; Cadosactis, Dan.; Cyathactis, Dan.; Korenia, Dan.; Aulorchis, Hertw.

It is possible to divide the Paractidæ into three sub-families, based on certain curious methods of mesenterial development. These families are:

(1) Paractinæ: In this sub-family the mesenteries of one and the same pair are about equally developed, or if any inequality occurs it is irregularly developed.

(2) Actinostolinæ: Here the mesenteries of the youngest cycles are usually unequally developed, one partner in each pair being larger than the other in such a way that that partner is always the larger which stands furthest away from the adjacent mesenterial pair of the next oldest cycle.

(3) Polysiphoniinæ: In this case the twelve oldest pairs of mesenteries are developed in the ordinary way, but the rest are peculiarly arranged. In each of the exocœls between the primary and secondary pairs the younger mesenteries have a bilateral arrangement, the youngest ones appearing in the middle of the exocœl. Each of these later pairs consists of one larger and one smaller partner, and the oldest of the larger partners are perfect as well as the primary and secondary mesenteries.

If this arrangement of sub-families be adopted, it is found that of the genera enumerated above Polysiphonia belongs to the third sub-family, Actinostola, Catadiomene, Stomphia and Ophiodiscus to the second, and the other genera to the first. There is not, so far as I can see, any objection to these three groups as sub-families, though I do not think they ought to rank as families. It may be found, however, when more work has been done on the subject, that between the Paractinæ and Actinostolinæ at least it will not be possible to draw a hard and fast line, the one merging into the other. This possibility has been suggested to me by a certain amount of work which I have done in connection with it, but I have merely the indication, and cannot as yet speak definitely one way or the other. Inequality of mesenteries, more or less regularly developed, is not confined to the Paractidæ, however.

PARACTIS, Milne-Edwards, 1857, tome i, p. 248.

Paractidæ (Paractinæ) with definite base. Column smooth, with a marginal collar; it may be thin-walled. Tentacles simple, in more than two cycles in the adult, their longitudinal musculature mesoglœal. Oral disc simple, its radial musculature mesoglœal. Perfect mesenteries with diffuse retractors; they may be fertile.

Species: P. papaver, Drayton in Dana, 1846, p. 143. (See Clubb, 1908, p. 3.)

A question which presents considerable difficulty is this: What is the genus Paractis? Milne Edwards (1857, tome i, p. 248) defines it on external characters as follows:

"Les Paractis ont, comme les Actinies proprement dites, le corps depourvu de verrues et les tentacules rétractiles; mais ils sont depourvu de tubercules calicinaux. On voit par conséquent que ce genre est avec les Actinies dans les mêmes relations que les Anemonia avec les Comactis. Ses tentacules sont presque éqaux et médiocrement nombreux. Enfin, le disque calicinal est circulaire."

An anemone eligible for this genus should therefore have smooth body without acrorhagi, a non-lobed disc, and retractile tentacles not exceptionally numerous. This description, however, would apply equally to different forms, not related to each other, and with quite different anatomy—e.g. to Epiactis and Stomphia. Since the definition does not indicate anatomy, the only way of getting at a more precise one is by referring to the actual species included by Milne-Edwards in the genus. The first four species which he mentions, in his order, are P. impatiens, P. monilifera, P. lineolata, P. papaver. P. impatiens has been anatomically described by McMurrich (1904) who found that it had acontia. Milne-Edwards can hardly have intended to include acóntia-possessing forms in his genus, or he would not have excluded it from his Actinines perforées, so McMurrich removed P. impatiens from it and called it Choriactis (see p. 547). P. monilifera seems to have some kind of marginal tubercles which make its position as a true Paractis untenable, and as far as I know its anatomy is undescribed. As to P. lineolata and P. papaver, both have been anatomically described, and either would serve as a genotype, but the two are quite different, and it is a case of choosing the right one.

Meanwhile Hertwig (1882, p. 41) set up a family Paractidæ, defined as possessing a mesoglœal sphincter and numerous perfect mesenteries. It is distinguished from any Sagartids, of course, by lack of acontia and cinclides. Of the two species lineolata and papaver, the second would come within the scope of Hertwig's family, the first would not, nor would it come within the Paractidæ as defined in this paper. It seems therefore the best policy to adopt P. papaver as the genotype, since it would cause great confusion and readjustment of names to alter the sense of Hertwig's family now. I have therefore drawn up my definition of Paractis to fit P. papaver, the anatomy of which was described by Clubb, 1908, p. 3. It may need a little modification for the inclusion of other species in the future, but should not be much widened. The possession of mesoglœal longitudinal tentacular musculature and of a collar, in addition to its numerous perfect mesenteries, are very definite features. The collar is certainly a feature absent from Milne-Edwards' definition, but it does not in any way contradict the sense of his genus; and since one is bound to define the genus on the basis of *one* of the orginally included species, P. papaver clearly fulfils the requirements better than either of the three mentioned before it. It seems best also to select the first *suitable* species named in order by the author of a genus, if the actual first one or more are ineligible. The acceptance of P. papaver as the genotype of Paractis will necessitate the removal from the genus of a number of other species which have been assigned to it from time to time before its boundaries were strictly enough limited, and which neither agree with each other or with P. papaver. This should really be an advantage, and tend to better represent the true relationships of the forms in question. Firstly, P. excavata, which is too aberrant a form as regards its tentacles to have ever been placed in Paractis, goes to Alloactis. P. lineolata and P. nivea probably find their place in Paranthus. P. tenuicollis requires a new genus (see Pseudoparactis). P. ignota is a Parantheoides and P. polaris apparently a Cymbactis. P. ferax goes to Isoparactis. Further notes regarding these species will be found under the genera mentioned. Other species assigned to Paractis, the anatomy of which is not yet known, await definite allocation.

An interesting form has been described by Stuckey (**84**) as Paractis fleuri. He does not describe the position of the longitudinal tentacular muscles, so that the form is hard to allocate. I do not think it comes under Paractis as here understood. Its sphincter is most remarkable—mesoglœal, but strongly circumscribed. Whether it will be the representative of a new genus, or whether it will fit into one of those here defined, I leave an open question for the present, pending further knowledge.

CYMBACTIS, McMurrich, 1893, p. 174.

PYCNANTHUS, McMurrich, 1893, p. 172.
PARACTIS as used by Clubb, 1908, p. 3, pro parte.

Paractidæ (Paractinæ) with definite base, which may be adherent or may form a mud-clasping cup. Column smooth but for capitular ridges in some cases; margin tentaculate and without a collar; wall variable, but may be very thick. Tentacles in more than two cycles in the adult, simple or somewhat thickened at the base, their longitudinal musculature mesoglœal. Oral disc simple, its radial musculature mesoglœal. Retractors diffuse. Older mesenteries may be fertile or sterile.

Species: Genotype, C. fæculenta, McMurrich, 1893, p. 174.

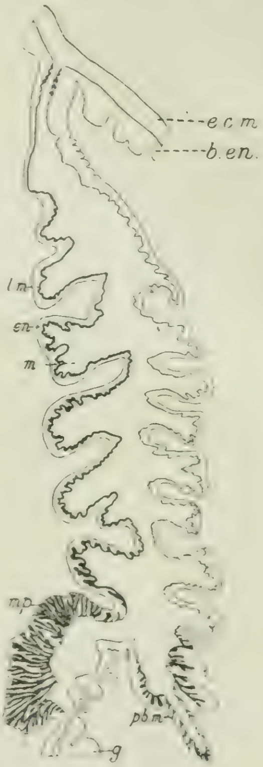
Other species: C. maliformis, McMurrich, 1893, p. 172.

C. actinostoloides, Wassilieff. 1908.
C. maxima, Wassilieff, 1908.
C. polaris, Clubb, 1908, p. 3.
C. gossei, Stephenson, 1918, B, p. 123.

McMurrich established a separate genus, Pycnanthus, for C. maliformis, which differed from Cymbactis as originally defined by possessing capitular ridges. As I have endeavoured to show above (p. 512) the development of these ridges is too variable to form a good generic character, and the genera are here fused.

C. gossei has irregular ridges in contraction which would probably disappear on expansion. C. polaris was originally assigned to Paractis by Clubb, but the revision of that genus as above makes its transference necessary, since it possesses no collar. The thickened tentacle-bases in C. gossei are not quite like the ordinary aboreal basal swellings found in some genera. Although the thickening may be least marked on the oral side, it generally affects the basal part of the tentacle all round, at least to some extent, and hardly seems definite enough to require the generic separation of C. gossei. At any rate I leave it for the present.

TEXT-FIG. 32.



Mesentery of Hormosoma scotti. *b.en.* Body-wall endoderm. *e.c.m.* Endodermal circular musculature. *en.* Endoderm. *g.* Gonad. *l.m.* Longitudinal musculature. *m* Mesoglœa. *m.p.* Mesoglœal processes for muscle. *pb.m.* Parietobasilar muscle.

HORMOSOMA, Stephenson, 1918, A, p. 29. (See Text-fig. 32.)

Paractidæ (Paractinæ) with definite base. Body-wall smooth (and in the only recorded species thick), without verrucæ or ridges, but with a prominent marginal collar which laps back over the column in expansion. Tentacles simple, in more than two cycles in the adult, their longitudinal musculature mesoglœal. Oral disc simple, its radial musculature mesoglœal. Mesenteries of all cycles fertile. The larger mesenteries with more or less circumscribed retractors, and in the only recorded species with curious ridges also, which support musculature (Text-fig. 32).

Species: H. scotti, Stephenson, 1918, A, p. 29 (see Text-fig. 32).

ALLOACTIS, Verrill, 1899, p. 206.

PARACTIS as used by Hertwig for P. excavata (1882, p. 41).

Paractidæ (Paractinæ) with definite base. Wall smooth, may be longitudinally furrowed, margin tentaculate, no collar. Tentacles of the adult in two cycles only, their longitudinal musculature reduced on the aboral side, probably even absent at the base, chiefly mesoglœal. Oral disc simple, its radial musculature principally mesoglœal. Mesenteries of all cycles fertile.

Species: A. excavata, Hertwig, 1882, p. 41.

Hertwig described the anemone above diagnosed as Paractis excavata. Later on Verrill (1899) showed that it was hardly correct to assign a somewhat curious form like this to the genus Paractis, which was meant for plain forms; he suggested for it a new genus, Alloactis, which I have here adopted. The peculiar constitution of the tentacles and their arrangement in two cycles only in the adult and its lack of a collar cut it off from Paractis as represented by P. papaver (see above).

ANTHOLOBA, Hertwig, 1882, p. 53.

METRIDIUM, Milne-Edwards, Hist. des corall., tome i, p. 252, pro parte.

Paractidæ (Paractinæ) with definite base. Wall smooth but for reticulations which may be present, margin unspecialised. Tentacles in more than two cycles in the adult, simple, very numerous, their longitudinal musculature (ectodermal to) mesoglœal. Oral disc lobed, its radial musculature chiefly mesoglœal. At least twenty-four pairs of perfect mesenteries, the primaries and some others sterile, the retractors diffuse, hardly specialised. Sphincter very long.

Species: Genotype, A. reticulata, Couthouy in Dana, 1846, p. 144. (= A. achates, Drayton, 1846.)

TEALIDIUM, Hertwig, 1882, p. 51.

Paractidæ (Paractinæ) with definite base. Column with papillæ or with vertical rows of verrucæ which may attack foreign bodies; with a circular swelling just below the crown of tentacles, in expansion at least. Tentacles simple, in more than two cycles except in small specimens, their longitudinal musculature ectodermal. Oral disc simple, its radial musculature ectodermal. Mesenteries all perfect and mesenteries of all cycles fertile except sometimes the directives.

Species: T. cingulatum, Hertwig, 1882, p. 51 (genotype).
T. cinctum, Stuckey, 1909, p. 389.

Further knowledge of this genus may perhaps result in its division into two genera, distinguished by structure and arrangement of the papillæ or verrucæ. I have included the statement that the tentacles are in more than two cycles except in small specimens, because in T. cinctum, which is of medium size, the tentacles are apparently in four cycles; and it seems very probable that the reason why there are only two cycles in T. cingulatum is that it is very small. It does not seem to be one of those cases (see p. 514) in which the tentacles never arrange themselves in more than two cycles however large the animal may grow.

PARANTHEOIDES, Carlgren, 1899, p. 28.

PARACTIS as used by McMurrich, 1904, pro parte.
DYSACTIS as used by Hertwig, 1882, p. 44, pro parte.

Paractidæ (Paractinæ) with definite base. Column smooth, margin indistinct. Tentacles simple, in more than two cycles in the adult, their longitudinal musculature ectodermal. Oral disc simple, its radial musculature ectodermal. The primary mesenteries may be fertile. Retractors diffuse, at least in P. ignota.

Species: Genotype, P. crassa, Carlgren, 1899, p. 28.
Others: P. ignota, McMurrich, 1904.
P. rhodora, Couthouy in Dana, 1846, p. 148. (See Hertwig, 1882, p. 49.)

Carlgren founded this genus for P. crassa, but there seems no doubt that the other species mentioned should also be included. P. ignota was assigned by McMurrich (1904) to Paractis, but it differs from that genus as now limited (p. 550) by having the longitudinal tentacular musculature ectodermal instead of mesoglœal. P. rhodora was included in Dysactis by Hertwig, but as McMurrich (1893, p. 169) showed, the genus Dysactis was probably erected for anemones with acontia, and therefore could not be used for Hertwig's species. Of the two species referred by Hertwig to the genus, one has been shown to be an Actinostola (McMurrich, 1893, pp. 169 and 209), and the other is rhodora, which may fairly be included in Parantheoides.

Sicyonis, Hertwig, 1882, p. 98.

Paractidæ (Paractinæ) with definite base. Column smooth; may be fairly thick; margin tentaculate. Tentacles in two cycles only in the adult, octamerously arranged, thickened all round at their bases, their longitudinal musculature mesoglœal. Each tentacle consists of a thickened proximal part with longitudinal musculature in its mesoglœa, and a thinner distal rim without longitudinal musculature and with an unusually large terminal opening; the longitudinal musculature, furthermore, is reduced on the aboral side of each tentacle. Oral disc simple, its radial musculature mesoglœal. In the only recorded species there are sixteen pairs of perfect mesenteries, and these are sterile and muscular, their retractors diffuse. Only the mesenteries of the last cycle bear gonads, and these are feebly muscular.

Species: S. crassa, Hertwig, 1882, p. 98.
S. elongata, Hertwig, 1888, p. 33.

There does not seem to be any very valid reason why this genus should not rank as a Paractid, judging by Hertwig's account of its anatomy. The tribe in which Hertwig placed it (Paractiniæ) has been discarded, and the genus exhibits the essential Paractid features. The curious structure and wide terminal openings of the octamerously arranged tentacles, and their limitation to two cycles only, although there are four cycles of mesenteries, distinguish it from Cymbactis, though some features of the tentacle-structure remind one of C. gossei. The large terminal openings of the tentacles do not seem to be a feature of any great importance, and it is difficult to know how far they may be due to state and method of preservation.

Pseudoparactis, n. nom.

Paractidæ (Paractinæ) (?) with definite base. Column divided into a thinner upper part or capitulum which is smooth, and a thicker lower part or scapus which may bear longitudinal ridges which may terminate rather abruptly above. Margin tentaculate, tentacles probably in more than two cycles in the adult. There is a weak sphincter in the capitulum, and apparently a second sphincter in the upper part of the scapus. Longitudinal musculature of tentacles ectodermal. Retractors diffuse.

Species: P. tenuicollis, McMurrich, 1904, p. 243.

It seems that the species described by McMurrich as Paractis tenuicollis (1904, p. 243) requires generic separation on account of the division of its body into scapus and capitulum, and the possession, apparently, of two sphincters, both mesoglœal. It is quite distinct from Paractis, both in this respect and in its ectodermal longitudinal tentacular muscle and lack of a collar. Unfortunately there is no statement made with regard to the number of its perfect mesenteries, so that whether it should be included here or in the Actinoscyphiidæ is uncertain.

Actinostola, Verrill, 1883.

Paractidæ (Actinostolinæ) with definite base, which may be adherent or may form a mud-enclosing hollow. Wall smooth or wrinkled or more or less tuberculated; it may be very thick; margin tentaculate. Tentacles in more than two cycles in the adult, hexamerously arranged, often short and stumpy, simple, their longitudinal musculature mesoglœal. Oral disc simple or somewhat undulate but not actually lobed, its radial musculature mesoglœal. Primary mesenteries at least sterile. Mesenteries hexamerous. Retractors diffuse. In the youngest cycles the mesenteries of each pair are unequally developed in such a way that the partner furthest away from the adjacent mesenterial pair of the next oldest cycle is the larger.

Species: Genotype, A. callosa, Verrill, 1882, pp. 224, 315. (See Carlgren, 1893, p. 71; McMurrich, 1893, p. 167.)

Others are: A. abyssorum (Dan.?), Carlgren, 1893, p. 66. (?Bunodes abyssorum, Dan.).

A. excelsa, McMurrich, 1893, p. 170.

A. pergamentacea, McMurrich, 1893, p. 171.

A. spetzbergensis, Carlgren, 1893, p. 76.

A. chilensis, McMurrich, 1904, p. 247. (See Clubb, 1908, p. 4.)

A. sibirica, Carlgren, 1901.

A. walteri, Kwietniewski, 1898.

A. crassicornis, Hertwig, 1882, p. 44.

?A. grœnlandica, Carlgren, 1899.

CATADIOMENE. n. nom.

ACTINOSTOLA as used by Carlgren, 1899, Wassilieff, 1908, and Stephenson, 1918, B, pro parte.

Paractidæ (Actinostolinæ) with definite base. Column wrinkled or more or less tuberculated or irregularly ridged; it may be very thick-walled; margin tentaculate. Tentacles in more than two cycles in the adult, hexamerously arranged, with swellings of the mesoglœa on the aboral side at the base; they may be short and stumpy and their longitudinal musculature is mesoglœal. Oral disc simple or undulate but not lobed, its radial musculature mesoglœal. Primary mesenteries at least sterile. Retractors diffuse. In the youngest cycles the mesenteries of each pair are unequally developed in such a way that the partner furthest away from the adjacent mesenterial pair of the next oldest cycle is the larger.

Species: C. atrostoma, Stephenson, 1918, B, p. 118.

C. carlgreni, Wassilieff, 1908.

C. intermedia, Carlgren, 1899.

It has seemed necessary to establish a new genus for those forms hitherto included in Actinostola, which differ from the typical forms in that genus by possessing basal swellings to their tentacles. Actinauge and Hormathia and other genera are mainly distinguished from each other in a similar way. The possession of the basal swellings is an apparently stable and well-marked feature and a very convenient generic distinction (see p. 516).

STOMPHIA, Gosse, Ann. Nat. Hist. 3, iii, 48, 1860, p. 221.

Paractidæ (Actinostolinæ) with definite base. Body-wall smooth and with tentaculate margin, but varying in thickness; it may be quite thin or quite thick or intermediate. Tentacles simple, in more than two cycles in the adult; their longitudinal musculature is mesoglœal, but there may be an ectodermal muscle-fringe as well as, and quite distinct from, the true mesoglœal musculature. Oral disc simple, its radial musculature mesoglœal. Tentacles and mesenteries frequently arranged octamerously (16–16–32). Perfect mesenteries sterile. Retractors well developed, diffuse. In the youngest cycle or cycles the mesenteries of one and the same pair are unequally developed in such a way that the partner furthest away from the adjacent mesenterial pair of the next oldest cycle is the larger.

Species: Genotype, S. churchiæ, Gosse, Ann. Nat. Hist., 3, iii, 48. (See Gosse, 1860, p. 222, Carlgren, 1893, p. 80, Stephenson, 1918, B, p. 126.)

Others: S. selaginella, Stephenson, 1918, A, p. 36.
? ? S. vinosa, McMurrich, 1893, p. 163.

I first described in 1918 (A, p. 36) a new Antarctic anemone under the name Cymbactis selaginella, one of my chief reasons for referring it to Cymbactis being its thick body-wall. But more recently I have been convinced (see p. 513) that there are too many grades of thickness in body-walls for this to be a valid feature for distinguishing genera. Since describing selaginella, also, I have had an opportunity of studying the type-species of this genus (S. churchiæ), and was surprised to find a striking similarity in structure between the two, which is sufficiently marked to make it evident that they belong to the same genus. My sections of S. selaginella show indications that the youngest mesenteries probably conform to the Actinostolinæ rule. They have in common certain features of general facies (when preserved); both have tentacles which can be short and thick and are generally arranged in three cycles on the 16–16–32 plan. There are sixteen pairs of perfect sterile mesenteries in both, and a comparative study of the mesenterial, tentacular and sphincter muscles reveals a good deal of similarity, though at the same time there is quite enough difference for *specific* distinction all the way through.

While referring to Stomphia I must correct an error into which I fell with regard to S. churchiæ in my 1918 B paper, p. 127. I there stated that after careful investigation of two animals I could not satisfy myself that the Actinostola-rule was carried out with any definiteness in Stomphia, although it held good for some sectors of the animal. I have had occasion to deal further with Stomphia since then, and sections of two whole individuals have proved that I was wrong and that the rule is indeed carried out almost exactly. I was misled before by relying too much on dissection.

I include very tentatively Paractis vinosa, McMurrich, as a possible Stomphia. It has the mesoglœal disc and tentacle-muscles and sixteen pairs of perfect sterile mesenteries, but on the other hand we do not know whether its small mesenteries conform to the Stomphia-rule, and its mesenterial musculature does not seem much like that of the other two.

OPHIODISCUS, Hertwig, 1882, p. 56.

Paractidæ (Actinostolinæ) with definite base. Column smooth, margin tentaculate. Tentacles simple, in a single corona, their longitudinal musculature mesoglœal and confined to the inner or adoral face of each tentacle. Oral disc simple, its radial musculature mesoglœal. The larger mesenteries are sterile and possess slightly developed musculature and the gonads are borne on the small unmuscular mesenteries of the youngest cycle. These young mesenteries are also unequally developed, in such a way that in each pair the partner furthest away from the adjacent mesenterial pair of the next oldest cycle is the larger.

Species: O. annulatus, Hertwig, 1882, p. 57.
O. sulcatus, Hertwig, 1882, p. 61.

I have restored the genus Ophiodiscus to the position in the Paractidæ originally assigned to it by Hertwig, because there does not seem to me to be adequate ground for placing it elsewhere. McMurrich has placed it in the Dendromeliidæ on the strength of a pseudo-tentacle which was supposed to have been connected with it—the possession of pseudo-tentacles being a diagnostic character of that family.

But I submit that there is no definite evidence that Ophiodiscus has any pseudo-tentacles, even though it cannot be actually stated that it has not. Hertwig says that there was *one* "pseudo-tentacle" enveloped in the same piece of cloth as *four* specimens of Ophiodiscus. In the first place this does not imply any connection between the pseudo-tentacle and the anemones. If I may mention an instance from my own experience, in a collection of anemones which I described, one bottle containing anemones had in it also a fine branched body which at first sight looked like a branched tentacle, but which on sectionising proved to be an Alcyonarian. My point is that there may have been no more connection between the Ophiodisci and the pseudo-tentacle than there was between my anemones and Alcyonarian. Again, there would surely have been more than one pseudo-tentacle among four Ophiodisci if it had really belonged to them, and Hertwig expresses his inability to find any pseudo-tentacle remains *on* the anemones. Another point is that the pseudo-tentacle in question had *circularly* arranged muscle-fibres in its *ectoderm*, and *longitudinal* fibres, also apparently muscular, in its *endoderm*. This arrangement is very difficult to account for if the pseudo-tentacle were an outgrowth of an anemone, in which case, according to ordinary rules, the *endoderm* should possess circular muscle and the ectoderm either no muscle-fibres or longitudinal ones. In Lebrunia danaë, which has genuine pseudo-tentacles, the endodermal musculature of the latter is, indeed, as one would expect, part of the endodermal circular muscle of the body-wall and its fibres run circularly.[1]

On these grounds I am inclined to think that the stray "pseudo-tentacle" had nothing to do with the Ophiodisci.

But be this as it may—and nothing but fresh and sufficiently well-preserved material can finally settle it—on its other characters Ophiodiscus is a Paractid, judged by Hertwig's account. It has pedal disc, numerous perfect mesenteries not divided into macrocnemes and microcnemes, mesoglœal sphincter, no acontia or cinclides, mesoglœal disc and tentacle musculature, and even exhibits the tendency, which I have mentioned elsewhere (p. 492), of the younger mesenteries to produce the gonads in higher anemones in its extreme form. It also exhibits an unequal development of the small mesenteries similar to that which prevails in Actinostola, Catadiomene and Stomphia. The absence of musculature on the aboral faces of the tentacles is a feature possibly foreshadowed by other Paractids in which the musculature is somewhat reduced on the outer sides of the tentacle-bases. So it seems that one must include Ophiodiscus in the Paractidæ unless definite evidence of pseudo-tentacles should come to hand, and even then that character alone *might* not be weighty enough, as against the sum of the other characters, to place it in a separate family. Certainly it could not go to the Dendromeliidæ.

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[1] This statement is made after consulting Pax, 1910, pp. 213–214. But I see that McMurrich, 1889 ('Journ. Morph.') describes what seems to be longitudinal musculature in the endoderm of the pseudo-tentacles of L. neglecta. He describes no circular fibres in the ectoderm, however, which resembles that of the body-wall but for the possession in parts of many nematocysts. I do not know how these conflicting statements can be reconciled, but Pax's description is the one in accordance with what one would expect on general grounds of anemone anatomy, and it is not impossible that the curiously made-up musculature described by McMurrich is an abnormality of that particular specimen.

POLYSIPHONIA, Hertwig, 1882, p. 63.

Paractidæ (Polysiphoniinæ) with definite base. Column smooth or somewhat nodular (and in the only known species thick-walled and vase-shaped), its margin tentaculate. All the tentacles have mesoglœal swellings on the outer side at the base, and their longitudinal musculature, like the radial musculature of the oral disc, is mesoglœal. The oral disc is twelve-lobed though the lobes are not very deep, and the tentacles are placed at its margin in two cycles only, the number of cycles not increasing as the animal grows. The tentacles are arranged in twelve triangular groups in connection with the lobing of the disc, the twelve groups being continuous with one another. The twelve tentacles representing the primary and secondary endocœls are the largest and are placed at the points of the twelve triangles nearest the mouth, i.e. in the depressions between the disc-lobes. The other tentacles decrease in size towards the apices of the disc-lobes. The outer-cycle tentacles represent the exocœls, the inner-cycle tentacles the endocœls. The twelve oldest pairs of mesenteries are perfect and arranged in the ordinary way found in Actiniaria, and include two pairs of directives, but the other mesenteries are peculiarly arranged. In each of the exocœls between the primary and secondary pairs the younger mesenteries have a bilateral arrangement, the youngest ones appearing in the middle of the exocœl. Each of these later pairs consists of one larger and one smaller partner, and the oldest of the larger partners are perfect as well as the primary and secondary mesenteries. The primary and secondary mesenteries probably sterile, the oldest of the others fertile.

Species: P. tuberosa, Hertwig, 1882, p. 63. (See Carlgren, 1918, pp. 13, 36, etc.)

In 1893 McMurrich (pp. 165, 209) included Polysiphonia in the genus Actinernus, relying apparently on the external similarity between the two. Carlgren (1918) has shown that there is no ground for fusing the two genera. The genus Actinernus as originally founded by Verrill has been broken up into two quite unrelated genera on anatomical grounds. The first of these is Actinernus proper (=Porponia, Hertwig), and is a member of the Endocœlactidæ; the second is Actinoscyphia and belongs to the Actinoscyphiidæ. Polysiphonia is a Paractid and related to neither of them. The main differences between the three genera are as follows: Actinernus has its younger mesenteries developed in the *endocœls* of the older ones, and each of these younger pairs has the longitudinal muscles of its partners facing away from each other as if they were directives; in Actinoscyphia and Polysiphonia the younger mesenteries develop in the normal way, i.e. in exocœls, and with the longitudinal muscles of the partners facing each other. Apart from this Actinernus has no sphincter and the other two have a mesoglœal one. Polysiphonia still further differs from Actinernus in that its discal, radial and longitudinal tentacular musculature is mesoglœal instead of ectodermal. Actinoscyphia in its turn differs from Polysiphonia by the possession of only six pairs of perfect mesenteries instead of a larger number; by the fact that its younger mesenteries are normally developed in cycles and do not appear bilaterally in twelve special regions of growth (see above definition of Polysiphonia); and by having ectodermal instead of mesoglœal tentacle-and-disc muscles. It is thus seen that but for a curious superficial and external resemblance to each other the three genera are unrelated, and Polysiphonia remains a good genus. The curious and unusual mode of appearance of the younger mesenteries in Polysiphonia offers an interesting parallel to a similar mode of growth exhibited by certain Endocœlactidæ not related. It is probably accounted for in both cases (as Carlgren has suggested, 1918, pp. 14–15) by the facts that the oral disc is lobed and the older tentacles at least have strong basal swellings of mesoglœa, so that newly-appearing tentacles have no room for normal development and must appear where they can, as the lobes grow, either between the bases of the lobes (Actinernus) or at their apices (Polysiphonia). Their appearing in this way carries with it the appearance of the younger mesenteries in a bilateral manner in zones related to the disc-lobes. It can hardly be the other way about (that the mesentery-development affects the tentacles), because in Synactinernus and Isactinernus, although the disc is lobed, the tentacles are small and have no basal swellings or only weak ones, and the mesenteries here develop normally in cycles. This explanation helps to elucidate the appearance of similar phenomena in unrelated forms.

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APPENDIX TO LIST OF LITERATURE.

The following list consists of references to literature which it has been neither possible nor necessary for me to consult personally, but which is mentioned in the foregoing paper in connection with some of the original descriptions of certain species listed after the generic definitions. In cases where the original description has not been consulted, one or more later and usually fuller descriptions of the species have been seen. These references are simply included for convenience in case they should be needed, and are copied from Andres, 1883, and other works. I cannot, of course, guarantee that the descriptions referred to will be found in the works quoted, but I have done my best to select the right references.

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XII. EXPLANATION OF PLATE 22.

Illustrating Mr. T. A. Stephenson's paper "On the Classification of Actiniaria."

PLATE 22.

A. Piece of a mesentery. *B.* Processes of mesoglœa bearing ectodermal muscle-fibres. *C.* Cilia. *D.* Diaphragm. *E.* Ectoderm. *EN.* Endoderm. *F.* Muscle-space in fig. 3; muscle-fibres in fig. 10. *H.* Small mesentery. *K.* Endodermal circular musculature. *L.* "Lip" of ectoderm. *M.* Mesoglœa; in fig. 7 points to muscle in the mesoglœa. *N.* Nerve-layer. *P.* Base of outer wall of a tentacle. *R.* Network of muscle-fibres and muscle-spaces in the mesoglœa. *T.* Thick-walled nematocyst. *Z.* Zooxanthellæ.

Fig. 1.—Transverse section of portion of body-wall of Sagartia miniata, to show a cinclis. Oc. 3, obj. $1\frac{1}{2}$.

Fig. 2.—Transverse section of portion of body-wall of S. miniata (same specimen as that from which fig. 1 was drawn), to show a cinclis. Oc. 3, obj. $1\frac{1}{2}$.

Fig. 3.—Longitudinal section of margin of body of S. miniata, showing the sphincter-muscle. Oc. 3, obj. $1\frac{1}{2}$.

Fig. 4.—Transverse section of an acontium of S. miniata. Oc. 3, obj. $\frac{1}{6}$.

Fig. 5.—Transverse section of the diaphragm of a cinclis of S. miniata. Drawn with $\frac{1}{12}$ oil imm.

Fig. 6.—Longitudinal section of portion of the wall of the physa of Peachia hastata to show a cinclis. Oc. 3, obj. $\frac{2}{3}$.

Fig. 7.—Longitudinal section of portion of the margin of Halcampa chrysanthellum, showing part of the mesoglœal sphincter. Oc. 3, obj. $\frac{1}{6}$.

Fig. 8.—Transverse section of a portion of the wall of a tentacle of Stomphia churchiæ. Oc. 3, obj. $\frac{2}{3}$.

Fig. 9.—Vertical transverse section of oral disc of Sagartia miniata. Oc. 3, obj. $\frac{2}{3}$.

Fig. 10.—Longitudinal section of margin of Aiptasia couchi. showing part of the mesoglœal sphincter. Oc. 3, obj. $\frac{1}{6}$.

Fig. 11.—Transverse section of part of an actinopharyngeal groove of A. couchi. Oc. 3, obj. $\frac{1}{6}$.

Fig. 12.—Diadumene schilleriana. Preserved specimen, about natural size.

Fig. 13.—Pelocœtes exul. Preserved specimen, about natural size.

XIII. Index to Genera.

A. Index to genera defined in this paper, and their chief synonyms. In each case the numbers printed in heavy type refer to the page on which the definition is to be found. Words in italics are synonyms.

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574 a

Ecdysis in a Teleostean Fish, Agriopus.

By

J. D. F. Gilchrist, M.A., D.Sc.,
Professor of Zoology in the University of Cape Town.

With 6 Text-figures.

Agriopus is a well-known fish of the Southern Hemisphere, and is recorded from South America, South Africa and Australia. On account of its striking appearance it attracted the attention of early travellers, and is first mentioned by Block under the name of Coryphæna torva. It has a mane-like dorsal fin and a long vertical forehead, from which it derives its local name "Horse-fish." A further characteristic, mentioned by all observers, is its soft, thick skin devoid of scales. This was specially noted by Cuvier and Valenciennes, who describe the skin of A. torvus as "épaisse et lisse comme un cuir bien préparé, sans aucune apparence d'écailles." They add that, among the specimens procured, there were some in which the skin was quite brown and raised in small wart-like prominences, so well marked as to suggest that these individuals represented a different species, though otherwise not dissimilar.

It may further be observed that among such brown specimens there are occasionally some in which the skin has apparently become detached, and hangs in ragged patches from the body. This might readily be put down to imperfect preservation, and consequent detachment of the epidermis, but the same condition was observed in specimens kept alive, and in another species, A. spinifer, kept alive for about two years in an aquarium tank, the skin was observed to become detached over the whole body and fins, leaving a brightly coloured new skin underneath.[1]

In order to obtain further information on this peculiar occurrence, which looks like a process of ecdysis, sections were made of the skin, and these revealed a condition which seems to be of more interest than a process of simple ecdysis, though this, so far as I am aware, is not known to occur in any fish.

The three Cape species (A. torvus, A. spinifer and A. verrucosus) were examined, and, though the process of casting the skin differs in these in some respects, the essential features are the same.

Agriopus torvus, Cuv. & Val.

In some individuals the skin has a bright, fresh appearance, with clearly defined broad bands of pigment along the body at intervals. In others the whole outer surface of the body and fins is of a dirty brown colour as if coated with a thick layer of diatomaceous growth. This layer may be of considerable thickness.

Sections of the brightly coloured skin (Text-figs. 1A and 1B), in which this layer does not appear to be present, show that it is there, but at an early stage of development. The normal elements found in the skin of fishes are present, there being a comparatively thin epidermal layer of about three to five cells in thickness. The lower cells are square, and rest on a well-marked basement membrane. The cells above this layer are rounded or polygonal in form, and are arranged more or less regularly in rows perpendicular to the epithelial surface. The outlines of all these cells are clearly defined. At the base of the epidermis there is a layer of black chromatophores, and scattered chromatophores are sometimes found in the epidermis itself. Sections of the skin in some parts of the body also show numerous saccular mucous glands within the epidermal tissue. The dermis underlying these elements is very thick and tough in this species of Agriopus, and there are no scales.

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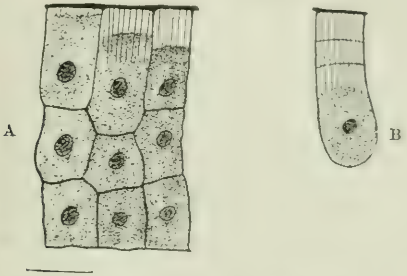
[1] 'Mar. Biol. Report, Union of South Africa,' 1914, p. 102.

The chief point of interest is the character of the outer cells, and the peculiar substance they produce at their free surface. The earliest stages of this substance can be seen in a fish in which the skin has been partly cast off, leaving certain parts of the body with the clear new skin exposed. The formation of this tissue must be very rapid, for already it is well marked in some places in the new skin, though at other parts there is little or no trace of it. For the purpose of ascertaining the minute structure of the outer cells and the substance they produce, very thin sections $(2\,\mu)$ were made, and stained in hæmalum—a stain which was found to be most suitable for the purpose of showing the structure of the cells and their cuticle-like product.

TEXT-FIGS. 1A AND 1B.



Section of skin of Agriopus torvus with little or no columnar development. [The degree of magnification is shown by the scale, representing $10\,\mu$, accompanying the figures. All figures are drawn by camera lucida, except fig. 6; fig. 2 with Zeiss C objective, the others with $\frac{1}{12}$ oil-immersion objective.]

In such sections (Text-fig. 1A) there is a fairly thick outer dark margin, which is continued down into the intercellular substance of the superficial epidermal cells. In a few cases the outer border of the cell was applied to this margin; in most, however, the cytoplasm was separated from the outer border, though still connected to it by fine fibrils. The space between the outer border and the cell was occupied by a clear substance, in which only the fibrils could be distinguished, and which was sharply marked off from the cytoplasm of the cell.

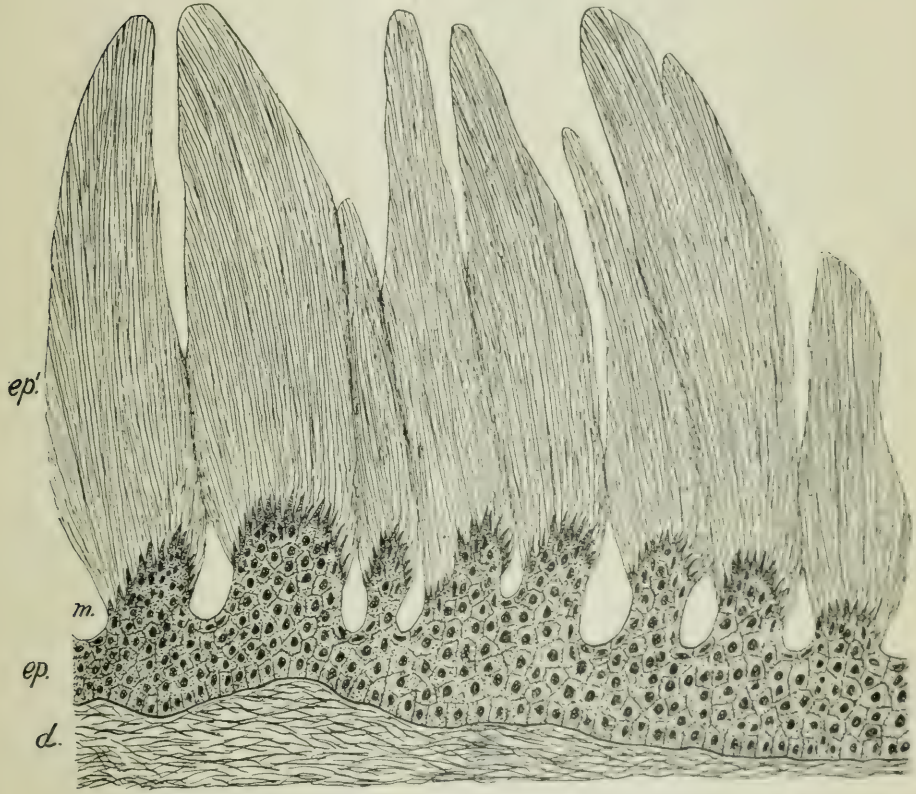
A later stage is evidently indicated by a cell which is further removed from the surface (Text-fig. 1 A). Here the fine fibrils from the outer border are again seen. In some cases they appeared to end abruptly at the cell, but in others they were continued down into the cytoplasm, almost to the nucleus, giving the distal portion of the cell a marked striated appearance.

Such stages were found alongside of each other, and at other places (Text-fig. 1 B) cells were found still further removed from the surface, so that the space between the outer border and the cell assumed the appearance of a clear short column traversed by longitudinal striations or fibres. Running across this column were what appeared to be transverse septa, not clearly defined and slightly curved, with the concave side toward the cell. The transverse septa varied in number from one to four according to the length of the column. They were not placed at regular intervals from each other (vide Text-fig. 3).

The wall or border between the columns is well marked, of a dark colour, and more clearly defined than the transverse septa. In some cases this wall was seen in such longitudinal sections to be broken up into short dark transverse streaks, which were to be seen more distinctly in transverse sections of the column (Text-fig. 5). The substance of the column in these sections was well marked off from the protoplasm of the cell, except in cases where the fibres penetrated its substance.

If sections of the skin be now examined in which the columns are further developed (Text-fig. 2), and can be seen macroscopically as the thick dark outer layer already mentioned, it is observed that this condition is brought about by the great development of the columns, accompanied by a modification in the outer epithelial cells. These are now more numerous, and are no longer arranged in rows perpendicular to the surface. There is a great variety in the characters of the outer cells; in some cases they are low and

TEXT-FIG. 2.



Section of skin of Agriopus torvus with fully-developed columnar layer. *ep.*, epithelium; *ep'.*, columns; *d.*, dermis; *m.*, mucus.

even flattened, as are also their nuclei, in a direction parallel to the epidermis; at other places they are narrow and greatly elongate in a direction at right angles to the epidermis (Text-fig. 3), being drawn out in long finger-like processes, which sometimes attain a length equal to the depth of the whole epidermis below them. The mode of termination of these long cells varies considerably. They sometimes pass without any clear line of demarcation into the substance of the column above them. At other times this demarcation is well marked, and the striæ of the columns are clearly continued down into their substance. At other places they are not only sharply marked off from the columns, but withdrawn from them, being separated by a well-marked space; in such cases the free end of the cell was often observed to present a vacuolated appearance. In some cases the columnar layer is entirely separated from the cells, and the process of ecdysis is apparently completed. The nuclei in these elongate cells show, in some cases, signs of disintegration. They appear sometimes as broken-up separate masses of chromatin, may assume a half-moon-like appearance, or appear like an irregular cross (Text-fig. 4).

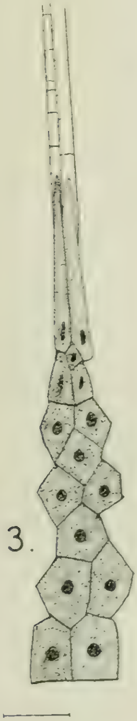
The elongate and flattened cells occurred in groups alternating with each other, the former giving rise to a fan-shaped structure, between which occurred depressions formed by the flattened cells. These depressions assumed the form of rounded spaces filled with homogeneous mucus (Text-fig. 2, *m.*).

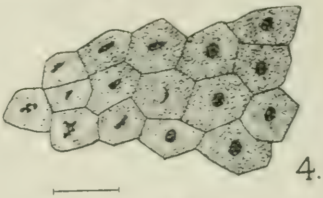
The consequence of this arrangement is that the outer columnar tissue is divided up into groups or bundles, which may appear as prominences on the outer surface of the skin. The columns composing these bundles are now greatly elongate. They are divided up by numerous transverse septa, but apparently are not otherwise interrupted, as some could be traced from the outer surface down to the corresponding epithelial cell. Very often of course the section does not pass through the length of the columns as shown in Text-fig. 2, but cuts them transversely or obliquely, when they appear as a number of rounded or oval areas.

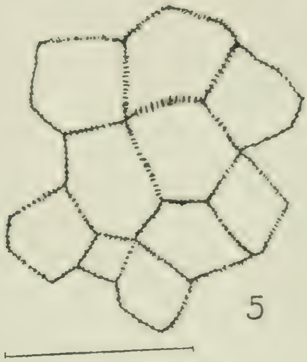
Transverse sections of the columns (Text-fig. 5) showed that they vary considerably in diameter in the same section ($2-7\,\mu$), and that they are on an average smaller in diameter towards the epithelium than near their outer extremities—

a fact which is doubtlessly associated with the narrower character and increased number of the superficial epithelial cells at this later stage. In one case in which actual measurements were taken, the average diameter of the distal end was 3μ and of the proximal end $2{\cdot}5\mu$.

TEXT-FIGS. 3, 4, 5, 6.







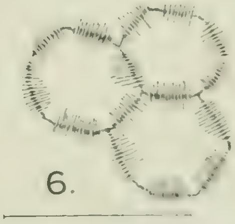


Fig. 3.—Enlarged view of part of the epidermis in above section. Fig. 4.—Oblique section of outer cells of epidermis at a later stage of development showing breaking up of nuclei. Fig. 5.—Transverse section of columns of skin of Agriopus torvus showing nature of their walls. Fig. 6.—Optical section of cast skin of Agriopus spinifer showing fibrils connecting columns.

The columns are polygonal in outline, so that they present a honey-comb appearance, but are not regular in shape.

In transverse sections further light is thrown on the nature of the walls of the columns. These may be thick or relatively thin, the thick walls being more marked towards the periphery of the bundle of columns. The walls present a dark appearance, being only slightly affected by staining reagents. The walls of the inner columns of the bundles are not so thick, and in thin sections they may be seen at places to consist, not of a single or double homogeneous wall, but of a number of closely-set dark lines, running from one column to the other across the wall, so that the column appears as if held together by slender fibres. Further details were difficult to make out on account of the slightly staining character of the substance and the minute size of the fibre.

How exactly the process of ecdysis is effected was not determined. It appears, in sections of the skin just cast, that the process is merely a pulling off of the columns, leaving the long, finger-like processes of the epithelial cell projecting. It is probable that this is associated with a breaking down of the superficial layers of epithelial cells, as indicated by the disintegration of their nuclei, which was observed only in these late stages.

Agriopus spinifer, Günth.

The skin of this species differs from that of A. torvus in having scales. These are deeply embedded in the dermis, and are sometimes broad and branched, and at the lateral line apparently fused together. They project as dark needle-like spines, which may or may not penetrate through the epidermis. In the latter case, where they approach the epidermis, they are covered by a proliferation of epidermal cells, presumably ameloblasts. The process of ecdysis is more marked in this species, as the whole of the outer columnar layer is thrown off at one time. This was readily observed in specimens kept alive at the Government Marine Laboratory near Cape Town, and individuals were occasionally got in the trawl with the loose skin only slightly attached to the body. The reason for this is apparently that in this species the active elongate cells of the epidermis are not grouped together in patches, alternating with patches of flattened cells, as in A. torvus. The shed skin is not so thick, and is clear and transparent, so that the outlines of the columns can be more distinctly seem. For this reason also the structure of the column, as seen in optical transverse sections, shows more details than in sections of the skin of A. torvus. The wall of the column was seen, as in A. torvus, to be made up of dark transverse striæ, and these were continued into the interior of the column, where they became very fine and faint in colour. In cases most favourable for observation they were seen to penetrate the column to a depth equal to about one-fourth of its diameter (Text-fig. 6).

Agriopus verrucosus, Cuv. & Val.

This species is distinguished from the last-named by having no bony projection in front of the eye. The skin is raised into rough projections, arising from the dermis. The structure of the skin appears to be similar to that of the other two species, but no suitably fixed material was available for examination.

The various species of Agriopus obviously require revision, and Jenyns is not sure but that his A. hispidus, from the coast of Chile, may not be identical with one of the three Cape species. Another species, A. alboguttatus, also from the Chilian coast, is of special interest in this inquiry, as the skin is described as covered with very minute horny tubercles. It is possible that here the columnar layer may have become solidified into such harder structures.

Nature and Origin of the Columnar Layer.

In view of these facts the question may now be considered as to the significance of the columnar tissue, which is apparently produced by the external epithelial cells. Does each column represent an excretion from the cells or a transformed and greatly elongate cell or cells? What is the significance of the longitudinal striation, and of the transverse septa?

That they do not represent mucus or other such excretion from the cell might be concluded from their straight course and well-marked walls, and that they represent transformed cells seems to be rendered certain from the structure of their walls, for both in longitudinal and transverse sections these were seen to consist of fibrils, passing from column to column, as seen in stratified epithelium generally, and also noted in some of the parts of the cellular epithelium of Agriopus itself.

With regard to the longitudinal striations, which are so well marked in the columns, it must be concluded from their development that they also represent such intercellular fibres, for they may be traced down into the protoplasmic substance of the cell.

The nature and origin of the transverse septa are not so apparent. They appear as distinct lines running across the column, less clearly defined, much thicker and more widely set apart than the fine fibrils, which pass along the column and into it. They are not so thick, however, as the walls of the column. The longitudinal fibres pass through them without interruption, and the most obvious explanation of their nature is that they represent cell-boundaries, like the walls of the column, and that therefore the interseptal spaces represent transformed epidermal cells. As against this, however, it can hardly be supposed that the sides of the column would be so straight and continuous if they represented a number of transformed cells of the epidermis. The fact also that the longitudinal fibrils or striæ can be traced without interruption through the whole length of the column is against this interpretation. Again, the interseptal spaces are very unequal, and in nearly all the cases very much smaller than the epidermal cell (cf. Text-fig. 3). The most probable explanation seems to be that these interseptal spaces represent segments of the striated end of the epithelial cell which have been successively cut off from it in its rapid growth, and that the septa represent modified fibrils or groups of fibrils.

The substance of the column, apart from the fibrils, is composed of a homogeneous structure, which does not readily stain. In some cases, however, the columns are mere empty spaces or vacuoles, their contents apparently having been removed.

The activity of these outer cells of the epidermis suggests a comparison with the activity of the inner cells—the ameloblasts—in the production of enamel. In mammals, as here, long columns or prisms are formed, separated from each other by distinct lines of demarcation, and crossed at intervals by transverse lines. In both cases these columns are produced from the free end of the epithelial cell. In the case of mammals, however, there is a collection of modified tissue—the stellate reticulum—not apparently represented here, and in this the substance of the enamel prisms is believed to arise and to be secreted by the ameloblasts on their outer side, so as to form the calcified prisms.

This stellate reticulum is absent in the formation of enamel in the case of Teleostean fishes—a subject which has been investigated by Tomes, Mummery and Carter. Tomes ('Phil. Trans.,' cxciii, B, 1900) describes the enamel organ of the Hake as consisting of an outer row of cells and an inner row which are the ameloblasts. In the next stage the ameloblasts have apparently disappeared, and in their place is a reticulated stroma of the full dimensions of the enamel which will ultimately appear. This reticulate stroma has a general appearance of fibrillation in the direction at right angles to the dental surface. The meshes of the reticulum are in section round or elongate. The rounded forms looked as if they might be the nuclei of the ameloblast cells, but it was concluded that they represent sections of rods or tubes. With regard to the fate of the ameloblast Tomes is uncertain. It at first greatly increases in length without losing its identity and its nucleus becomes less conspicuous, but it could not be made out whether the ameloblast cells go to form the rods and tubes of the stroma, the interstitial material being formed outside or between them, or whether the whole cell breaks up into the reticular tissue. Tomes concludes that the enamel is produced by calcification of the reticulate stroma.

Mummery ('Phil. Trans.,' ccciv, B), in a paper on "The Tubular Enamel of the Sparidæ and Labridæ," apparently accepts Tomes' view of the products of the ameloblasts, but Carter ('Quart. Journ. Micr. Sci.,' vol. 63), from a further study of the enamel organ of the Hake, arrives at the conclusion that some at least of the tubular structures are really the nuclei of the ameloblasts; others may be vacuoles in a "fibrillar cytomitoplasm, whose fibres run fairly parallel with the long axis of the cell."

We are not directly concerned here with the origin of the enamel, whether by a transformation of cells or a secretion from their ends, but only with the fact that the lower cells of the epidermis become elongated, and give rise to a fibrillated substance. These long fibrillated cells seem to be comparable with the long fibrillated cells or columns which arise on the outer surface of the epithelium of Agriopus, and the fibrils may in both cases be interpreted as no new structure in epithelial cells, but the ordinary connecting fibrils of stratified epithelium, greatly drawn out with the elongation of the cell. Fibrils running across the column are also seen in Agriopus, and it would be instructive if these were also found to occur in the elongate cells of the enamel organ of Teleosts.

SUMMARY.

(1) The Teleostean fish Agriopus appears to shed its skin at certain times.

(2) This shed skin consists of numerous elongate columns, which are striated longitudinally and are continuous with the outer epidermal cells.

(3) The walls of the column are composed of transverse striations, which are prolonged for some distance into the substance of the column.

(4) The walls of the cells of the epidermis are composed of such fibrils or striæ, and it is concluded that the columns represent a greatly elongate and transformed part of the outer cells.

(5) In the columns are also transverse septa or lines, the nature of which is not evident.

(6) The striated columns are compared with certain features in the enamel prisms of mammals, and with the striated elongate cells of the enamel organ of Teleosts.

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